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Approximately 15% of the added mercury was removed from the water. The community components acquired very high concentrations of mercury, although in most cases the levels in these were not a linear function of the water levels. Concentrations in invertebrates decreased most rapidly after mercury inputs were stopped while the sediment levels decreased most slowly.

Periphytic algae showed several treatment responses including total inhibition, inhibition of certain life stages, possible stimulation of certain life stages and morphological alterations. One species was found only in the treated channels and disappeared when mercury inputs were stopped.

The diversity and evenness of benthic insect communities were affected by mercury treatment.

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August 1976

FATE AND BIOLOGICAL EFFECTS OF MERCURY INTRODUCED INTO ARTIFICIAL STREAMS

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ABSTRACT

Mercuric ion was continuously input to artificial stream channels to provide water concentrations of 0.01, 1.0 and 5.0 µg/l. Channel components were periodically sampled for total mercury analyses. The effects of mercury on the algal components of the periphytic communities and on the benthic insects were determined. The sampling program continued one full year after mercury inputs were stopped.

Approximately 15% of the added mercury was removed from the water. The community components acquired very high concentrations of mercury, although in most cases the levels in these were not a linear function of the water levels. Concentrations in invertebrates decreased most rapidly after mercury inputs were stopped while the sediment levels decreased most slowly.

Periphytic algae showed several treatment responses including total inhibition, inhibition of certain life stages, possible stimulation of certain life stages and morphological alterations. One species was found only in the treated channels and disappeared when mercury inputs were stopped.

The diversity and evenness of benthic insect communities were affected by mercury treatment.

This report was submitted in fulfillment of Project Number R800510 by the University of Georgia under the sponsorship of the Environmental Protection Agency. Work was completed as of April 1, 1975.

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SECTION I

INTRODUCTION

The toxic effects of Hg and its compounds on both plants and animals have been known for a long time. It is only within the last twenty-five years, however, that the extent of man's uses of this element, and the actual and potential problems resulting from his uses, have been recognized. The history of Hg, its chemical and physical properties, sources, uses, and toxicological effects as related to current problems have been reviewed in a number of recent books (Jones, 1971; D'Itri, 1972; Friberg and Vostal, 1972; Hartung and Dinman, 1972), dissertations (Curtis, 1974; Ferens, 1974), and review papers (Nelson, 1971; Wallace et al., 1971; Peakall and Lovett, 1972; Putman, 1972; Saha, 1972; Lepple, 1973).

At present, high-level environmental releases of Hg from point sources such as chlor-alkali plants and paper pulp operations have been virtually eliminated. Releases due to agricultural usage of organomercurials have also been greatly reduced. It is unlikely that we will see another Minimata Bay type incident, although the poisoning of man by the misuse of grain treated with mercurials remains a possibility. The two major problems still facing man with respect to mercury are: (1) what to do about the areas already contaminated by large releases in the past and, (2) what are to be the consequences of the continuing large-scale diffuse releases of Hg from the burning of fossil fuels and the discarding and incineration of Hg-containing products.

Since the recognition that Hg releases of any kind may result in ecosystem alterations and even poisoning of man himself, a great deal of research dealing with this metal has been initiated. The majority of the studies have been surveys or experiments of short duration involving relatively high Hg levels and/or very simple biological systems, or performed in existing contaminated areas without adequate controls and replication. These studies have demonstrated that:

(1) Mercury is universally distributed and occurs in small amounts in all components of the biosphere. The inability to find Hg in a sample is probably a result of the analytical techniques used.

(2) There is a natural biogeochemical cycle in which Hg circulates between living organisms and the environment. Inputs to the natural cycle include releases from the earth's crust by the weathering of Hg-bearing rocks, volatilization of Hg from covered deposits, leaching by ground water, and releases associated with volcanic activity and thermal springs.

(3) Mercury can exist in three general forms (organic compounds, inorganic compounds and free metal) which differ greatly in their physical and chemical properties and, therefore, their behavior in biological systems. These forms have different accumulation and clearing rates, sites and physiological effects on living organisms. All possible conversions between forms can and do take place in natural systems.

(4) Man adds to the Hg load of the environment in several ways. Point source releases have caused localized problems including human deaths. Agricultural use of mercurials has resulted in wildlife losses. Although point source and agricultural releases have been almost entirely eliminated in the past few years, contaminated aquatic areas still exist and fish from these areas remain unfit for human consumption. There continue to be extremely large releases of Hg wherever fossil fuels are utilized.

(5) From the standpoint of human health, methyl Hg compounds are the most important because this is the form that tends to accumulate in food organisms. Methyl Hg is almost completely absorbed in the gut of mammals, is very slowly eliminated, has a great effect on the central nervous system at low levels, and easily passes through placental membranes.

(6) Relatively low levels of all forms of Hg may be toxic to aquatic organisms. The toxicity is a function of species and water quality.

In spite of the recent surge of Hg-related research, several important questions have not been fully answered. The site of methylation of the Hg that occurs in fish as methyl Hg has not been clearly demonstrated. The effects of long-term exposures of biological systems to slightly elevated Hg levels have not been adequately investigated. The rates of recovery to be expected from contaminated systems are not clearly defined. And the maximum levels that will not cause environmental alterations or the contamination of human food resources have not been determined. The research program reported here was directed to the last three points as pertaining to aquatic systems. More specifically, the artificial stream research program had the following general goals: (a) to determine the fate of Hg introduced on a continuous basis at low levels into biologically complex aquatic communities over a long period of time, (b) to determine the fate of Hg remaining in the systems after the inputs were stopped, (c) to assess the impact of the added Hg on the communities in the systems and, (d) to compare results obtained from the artificial streams with data from other sources in order to evaluate the artificial streams as a potential tool for the future routine screening of environmental pollutants.

SECTION II

CONCLUSIONS

1. Water levels of only 0.01 μg/l Hg^{++} above ambient resulted in Hg levels significantly greater than background in components of the communities that developed in the artificial streams.

2. At 1.0 and 5.0 μg/l Hg^{++}, very high Hg concentrations accumulated in the biota and sediments of the treated channels although, except for insects and macrophytes, the relationship between water levels and the levels measured in the community components was not linear.

3. In a simple food chain (periphyton-midge larvae-mosquitofish) no biomagnification was observed.

4. At dosing levels of 1.0 and 5.0 μg/l, approximately 15% of the Hg was removed from the water during the two-hour retention time of the channels. Approximately 33% of the Hg leaving the 1.0 μg/l channels and 21% of that leaving the 5.0 μg/l channels was attached to microscopic particulates. No diurnal Hg uptake patterns were observed, indicating uptake was by passive adsorption in the channels.

5. The major loss of fixed Hg from the channels was with exported macroscopic matter. These losses were erratic and very much affected by weather conditions.

6. A loss of elemental Hg from the channels at the air-water interface was observed and this loss rate was linearly related to water concentrations of Hg and not to sediment levels. The release of Hg from the channels appeared to be related to the diurnal photosynthetic activities of channel communities. The maximum releases measured were always less than 1% of the Hg present in the water.

7. After Hg inputs were stopped, there were major differences between the clearing rates of Hg from the various channel components. The sediments showed greatest Hg retention ($T_{1/2}$ = 7.85 years, $C.I._{95}$ = 5.6-13.0 years in 1.0 μg/l channels, $T_{1/2}$ = 8.67 years, $C.I._{95}$ = 6.11-15.0 years in 5.0 μg/l channels), and an odonate nymph the least ($T_{1/2} \simeq 15$ days). One year after the Hg inputs were stopped, all system components analyzed still had elevated levels relative to the controls.

8. During the project period the channel communities were continuously changing, and no steady-state systems developed. There was continual downstream expansion of the macrophyte populations, a constant rearrangement of insect and algal populations, and a continuous influx of new plant and animal species into the channels.

9. Periphyton colonization was retarded in the Hg-treated channels with respect to controls although greater biomasses eventually developed in these channels along with decreased species diversities.

10. Periphytic algae were affected by Hg treatment in a variety of ways including:

    (a) inhibition during all parts of the life history.

    (b) inhibition during the reproductive and stimulation during the vegetative part of the life history.

    (c) morphological alteration without a significant effect on growth, and

    (d) complete restriction of one species to treated channels with the greatest densities occurring at the highest Hg concentration.

    Short-term studies using continuous-flow chambers confirmed the growth retardation observed for some of the stream algae under more controlled conditions.

11. The most abundant macrophyte, Juncus diffusissimus, showed a negative response to Hg treatment.

12. Insect densities showed no consistent responses to the Hg concentrations tested; however, the diversity and evenness of the benthic insect communities were significantly lowered at the highest treatment level.

SECTION III

RECOMMENDATIONS

1. The present drinking water standards regarding Hg should be carefully reviewed since currently permitted levels can cause ecosystem changes, and also result in fish with concentrations too high for human consumption.

2. Additional long-term experimental studies with replicable complex microcosms should be done with both Hg and other environmental contaminants. Treatment levels should be realistically low, and a variety of environmental conditions (geographical areas) utilized. Interactions between contaminants should be considered.

3. Modifications of the artificial stream facility used in this project should be made so as to decrease the variability between channels treated alike.

SECTION IV

EXPERIMENTAL DESIGN AND SITE DESCRIPTION

EXPERIMENTAL DESIGN

The basic experimental design was to input water of constant quality at a constant rate into six artificial stream systems. After biological communities became established in the channels, fish were to be added, followed shortly by the continuous introduction of Hg^{++} to establish water levels of 0.01 and 1.0 μg Hg/l. The Hg inputs were to be continued for one full year, during which time samples of water, lining material, biota and sediments were to be taken and analyzed for total Hg content. During this same period, the structures of select portions of the channel communities were to be determined by detailed species counts of periphytic algae and benthic insects. Estimates of community productivity and respiration were to be made from the continuous monitoring of upstream-downstream changes in dissolved oxygen and pH. All sampling programs were to be continued for one additional year after the Hg inputs were terminated.

The experimental program deviated from the original plans as work progressed. Early data indicated certain sampling programs were not producing useable information and these were discontinued. Changes in sampling techniques were made necessary by changes in the biological communities. Analytical procedures were simplified to allow for more efficient handling of samples. And the original Hg dosing schedule was changed when it became evident that the 0.01 μg/l water concentrations were not providing contamination levels sufficiently high to permit the study of the clearing of Hg from the channels receiving this input.

ARTIFICIAL STREAM FACILITY

The artificial stream complex is an Environmental Protection Agency (EPA) facility located on the Energy Research and Development Administration's (ERDA) Savannah River Plant (SRP), a 507-km^2 reserve that includes portions of Aiken, Barnwell and Allendale Counties in South Carolina. The six concrete block channels (Figure 1) are 91.5 m long, 0.61 m wide and 0.31 m deep with concrete block pools (1.5 m X 3.0 m X 0.92 m) at both ends of each stream. The channels were constructed on a level concrete slab

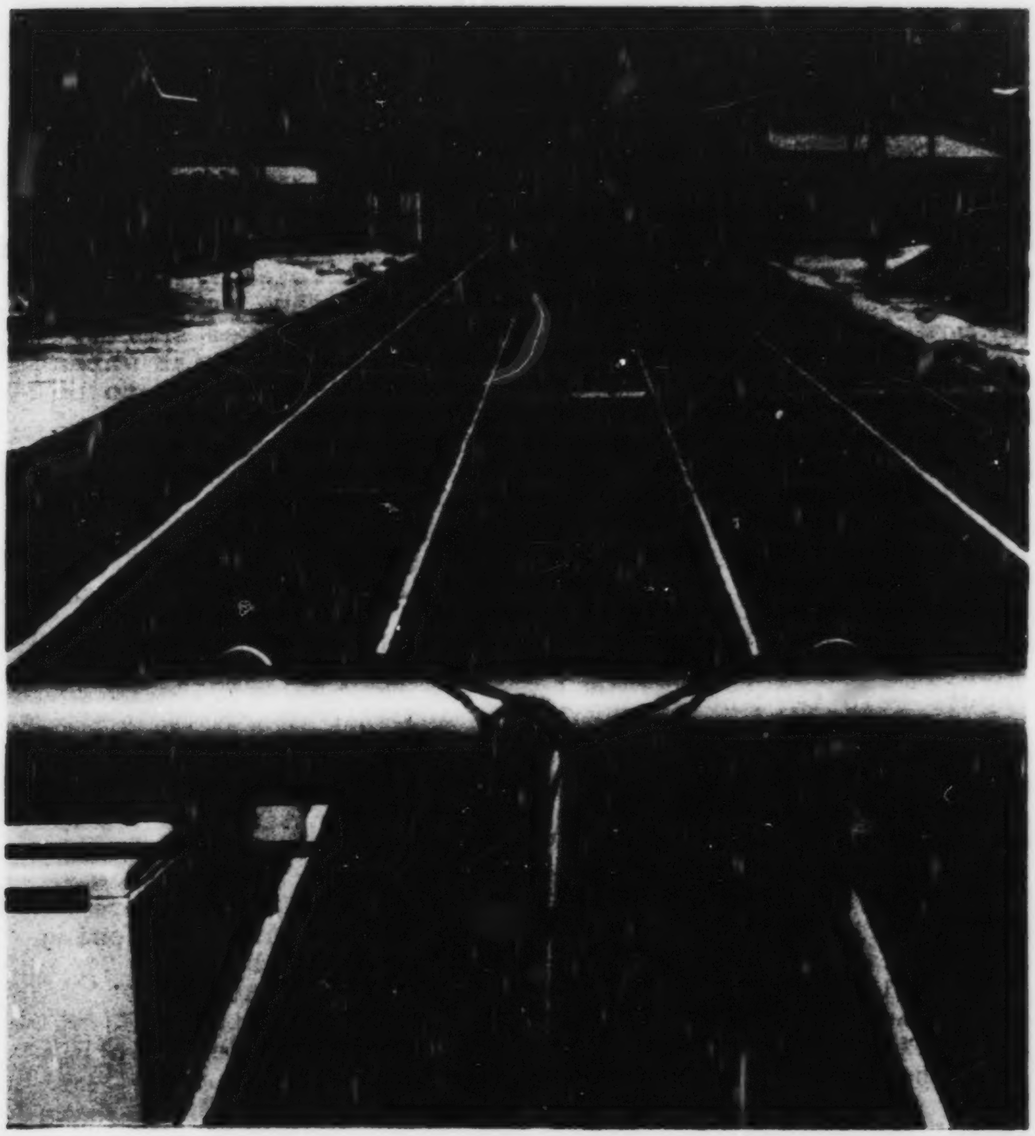

FIGURE 1. ARTIFICIAL STREAMS. IN THIS PHOTOGRAPH TAKEN FROM THE HEAD POOLS, CHANNEL 1 IS ON THE RIGHT AND CHANNEL 6 IS ON THE LEFT. THE PLASTIC STRIPS AND FISH-HOLDING CAGES CAN BE SEEN.

which was once a building foundation. For this study, the pools and channels were lined with a 0.05-cm-thick black polyvinyl chloride (PVC) film.

CHANNEL WATER AND SUBSTRATE

Water for the channels is pumped from a well located near the facility. The chemical composition of this water, based on analyses made over a 10-year period by personnel of the E. I. duPont Corporation, is presented in Table 1. During the period of this study, the average pH of the well water was 4.6 rather than the 5.3 shown in Table 1. The water was treated at the stream site by passage through limestone-filled tubes (Figure 2) in order to decrease the free CO_2 content and increase the hardness and pH. The limestone, obtained at the beginning of the project from a quarry in Rome, Georgia, was changed approximately every 30 days. An analysis of the limestone as provided by the supplier is given in Table 2. Figure 3 shows the average hardness of the water input to the pools at the head of the channels as a function of time. In addition to this time variation in water quality between limestone changes, there were measurable differences between channels during the latter part of the project. This was due to leaks that developed in the head pools, which made it necessary to input water at different rates into these pools in order to maintain desired flows into the channels.

Periodic measurements of dissolved organic matter, nitrate nitrogen, orthophosphate, conductivity, pH, temperature and dissolved oxygen were made on samples of the influent water. The dissolved organic carbon levels were always less than 0.5 mg/l, the nitrate-N levels averaged 38 µg/l (Ferens, 1974), and the orthophosphate levels averaged 76 µg/l (Ferens, 1974). The conductivity decreased from approximately 30 to 22 µmho/cm and the pH decreased from 6 to 5 during the period between limestone changes. The average temperature of the incoming water varied seasonally but only slightly about the mean of 23°C. Water entering the channels was generally saturated with respect to dissolved oxygen.

The rate of water entering each channel was controlled by a manual valve located just before the limestone-filled treatment tubes. Flows were checked daily against gradations on a V-notch weir separating the pool at the head of each channel from the channel itself.

FIGURE 2. LIMESTONE-FILLED TREATMENT SYSTEM.

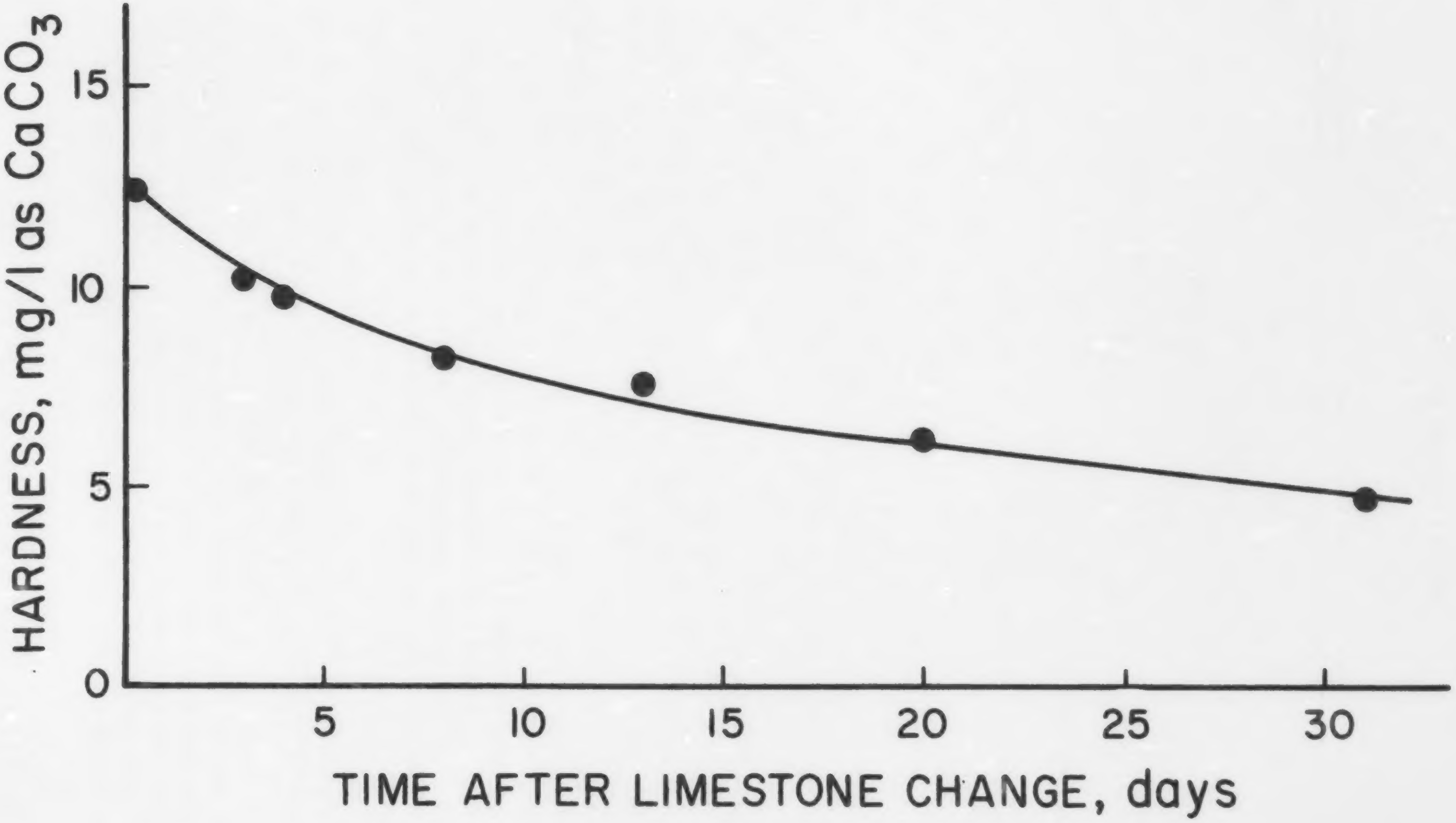

FIGURE 3. AVERAGE CHANGE IN CHANNEL WATER HARDNESS BETWEEN LIMESTONE CHANGES.

Table 1. WELL WATER QUALITY 1963-1973
(After Ferens, 1974)

Analysis	N	$\bar{X}$	CV
Temp	4	22.8°C	15%
pH	13	5.3	7
Cond	11	19.7 µmho/cm	18
TDS	8	13.1 mg/l	42
DO	5	7.1	18
Hardness (EDTA)	12	6.56 ($CaCo_3$)	67
Mg^{++}	8	1.87	88
Ca^{++}	7	0.63	108
K^{+}	7	0.47	17
Na^{+}	10	2.48	115
Fe^{+++}	10	0.04	57
Cu^{++}	8	0.09	71
HCO_3^-	8	0.39	149
Cl^-	8	2.59	79
$SO_4^=$	8	1.90	71
SiO_2	9	7.82	70

In September of 1971, washed builders sand was distributed in the channels to a uniform depth of 0.05 m and the water input started. Flows of 95 l/min into each channel, as measured by V-notch weirs between the head pool and channels, were maintained until February, 1975, except for several short intervals of reduced flow due to water pump failures. Stainless steel end plates between the channels and tail pools were adjusted to maintain a water depth of 20 cm over the sand. With these settings, mean retention time of the water in the channels was 2 hr and the average water velocity was 0.013 m/sec.

Water leaving the treated channels was passed through beds of shredded rubber, (Figure 4) a waste product of commercial tire recapping operations. Results of water analyses made on a number of different occasions show that at least 50% of the Hg leaving the channels was taken up by the rubber particles. Laboratory uptake studies have shown that a much higher removal of Hg is possible if contact between the water and rubber is complete (Kania and Beyers, 1974). The use of shredded rubber for Hg removal

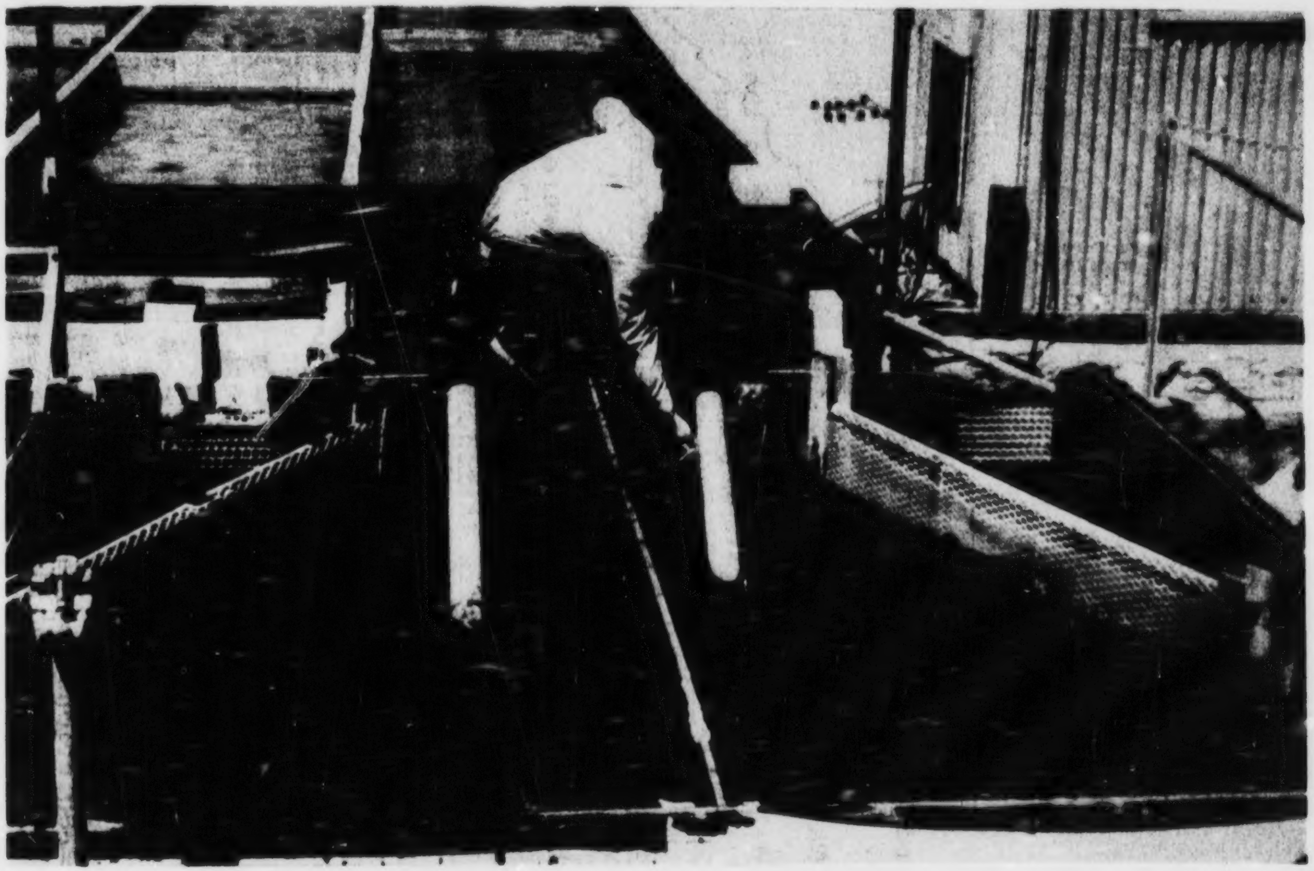

FIGURE 4. MERCURY RECOVERY SYSTEM. EFFLUENT WATER ENTERED POOLS FILLED WITH SHREDDED RUBBER OBTAINED FROM A TIRE-RECAPPING PLANT.

Table 2. LIMESTONE COMPOSITION
(Analysis provided by the quarry)

CaO	52.73%
MgO	1.70
SiO_2	3.42
Fe_2O_3	0.25
Al_2O_3	0.33
Na_2O	0.10
S	Trace

was suggested by Edwin D. Russell of the E. I. duPont Corporation.

CHANNEL BIOTA

The biological communities that became established in the channels developed from organisms carried in by the wind or by animals visiting the stream facility. A variety of microorganisms was probably added with mosquitofish, Gambusia affinis, that were introduced at two different times in the channels.

Mosquitofish were seined from a nearby pond and were acclimated for two weeks in artificial ponds receiving water similar in quality to that input to the channels. In April, 1972, 400 fish were introduced into each channel and 40 into each of two 51-cm X 30.5-cm stainless-steel screen cages suspended 10 m from both ends of each channel. Fish were confined to the channels by coarse stainless-steel screens. Caged fish were fed three times a week with a commercial tropical fish food (Bi-O-Rell). In May, 1973, all fish remaining in the channels and cages were removed for analyses and new animals introduced, again 400 into each channel, 40 into each cage.

MERCURY INPUT

Mercury was pumped continuously into the streams with a four-channel peristaltic tubing pump (Sage Instruments Model 375). Solutions for each channel were made daily with the concentrations adjusted to compensate for changes in pump characteristics. The concentrated solutions from which the daily solutions were prepared were made by dissolving dried reagent grade $HgCl_2$ in 2% (vol/vol) HNO_3 to provide Hg^{++} levels of 0.1 mg/l, 10 mg/l, and 20 mg/l. Measured amounts of these stocks were diluted with well water to 2 l in a volumetric flask and transferred to polyethylene bottles connected to the input tubes of the pump. Based on the volume removed, and the elapsed time between changes, a new concentration for the input mixture was calculated and new solutions prepared.

Mercury inputs to four of the six channels were begun on May 15, 1972, so as to establish water concentrations of Hg^{++} at 0.01 µg/l above ambient in two channels (3 and 6 as counted left to right looking upstream), and 1.0 µg/l in another two channels (2 and 5). Channels 1 and 4 were used as controls. Since the six channels are structured as three pairs (Figure 1), the dosing arrangement was chosen so that there was a northern and a southern exposed surface for each treatment.

On August 1, 1973, the input levels to channels 3 and 6 were increased to 5.0 µg/l. This was done because it was obvious that the 0.01 µg/l dosing levels were not providing high enough Hg concentrations in the channel systems to permit the study of the removal of this metal after inputs were stopped. All Hg inputs were stopped on January 29, 1974, although routine sampling continued until February 10, 1975, when the study was terminated.

SECTION V

METHODS

SAMPLING PROCEDURES

Water

Prior to August 1, 1973, only occasional water samples were taken for Hg analyses, and then only from the channels receiving an input of 1.0 μg/l. Mercury in the water from the channels receiving inputs of 0.01 μg Hg/l was below detection limits of the techniques used. After August 1, 1973, the date of the dosage change from 0.01 μg/l to 5.0 μg/l, samples were taken routinely from two locations in each channel (including controls) and also from two regions of the effluent channel leaving the stream facility. Sampling stations were located approximately 6 m from the Hg input tubes at the heads of the channels and 1 m from the downstream ends of the channels. Samples of the effluent water leaving the tail pools were taken just below where the individual outflows joined into a common stream and also 100 m farther downstream.

The samples were taken by submersing a nitric-acid-rinsed 250-ml Erlenmyer flask. The contents of the flask were checked for visible particulates and a new sample taken if particles were present. A clear sample was transferred to a nitric-acid-rinsed polyethylene bottle containing 2.5 ml of concentrated HNO_3.

Air

In the initial stages of the project, no attempt was made to measure Hg losses from the channels at the air-water interface. Based on reports by Fagerstrom and Jernelov (1972) and Jernelov and Lann (1973), no losses were expected from the acidic waters (pH 5-6) of the channels because monomethyl Hg was supposed to be formed rather than the more volatile dimethylmercury. A slight increase in Hg levels of some components of the control channels, however, indicated a transfer might be occurring from the treated systems. The possibility of leaks was remote since each channel was covered with a single continuous sheet of PVC film and there was no pressure differential across the wall separating adjacent channels. Therefore, the possibility of air transfers was investigated.

Six air sampling systems, as shown in Figure 5, were constructed and the glass boxes, which covered 0.187 m^2, suspended at the midpoint of the channels by stainless-steel straps. Air was drawn from under the boxes at the rate of 0.47 l/s, dried with $Mg(ClO_4)_2$ and then passed through gas-scrubbing bottles containing 100 ml of a 10% (vol/vol) H_2SO_4, 1% (wt/vol) $KMnO_4$ solution. On three occasions, two scrubbing bottles were connected in series. Analytical results showed that 98% of the Hg was collected in the first scrubber.

To define the form of the Hg at the air-water interface, traps consisting of silver-plated copper turnings were placed between the drying tube and the gas scrubber containing the acid permanganate. On three occasions, adjacent boxes were placed in channel 6 (5.0 µg/l), one with and one without the Ag-Cu trap and the air sampled for a two- or three-hour period.

During routine sampling, air samples were taken in the early afternoon on clear days.

Sediment

Several different techniques were used to obtain sediment samples. Early in the project, a coring device, which removed frozen cores 14 mm in diameter, was used to determine the vertical distribution of Hg in the sediments. The frozen cores were sectioned into three zones and analyzed. This method of sampling was discontinued when it became obvious that the Hg in the sediments was entirely in the organic component present as a thin layer on top of the sand.

As described in a following section, the processing of pyrex dishes that had been placed in the channels for invertebrate sampling resulted in a separation of the organic portion of the sediments from the sand. Both of these components were analyzed for Hg and the organic content of the organic component was measured.

During the last year of the study, samples were taken with a plexiglass tube 5.5 cm in diameter and 40 cm long. The tube was pushed vertically through the sediments until it touched the channel bottom. The material was retained in the tube by hand as it was transferred to a container for transportation. In the laboratory, the macrophytes were removed and saved. The core material, free of plants, was blended for several seconds and the suspended sediments

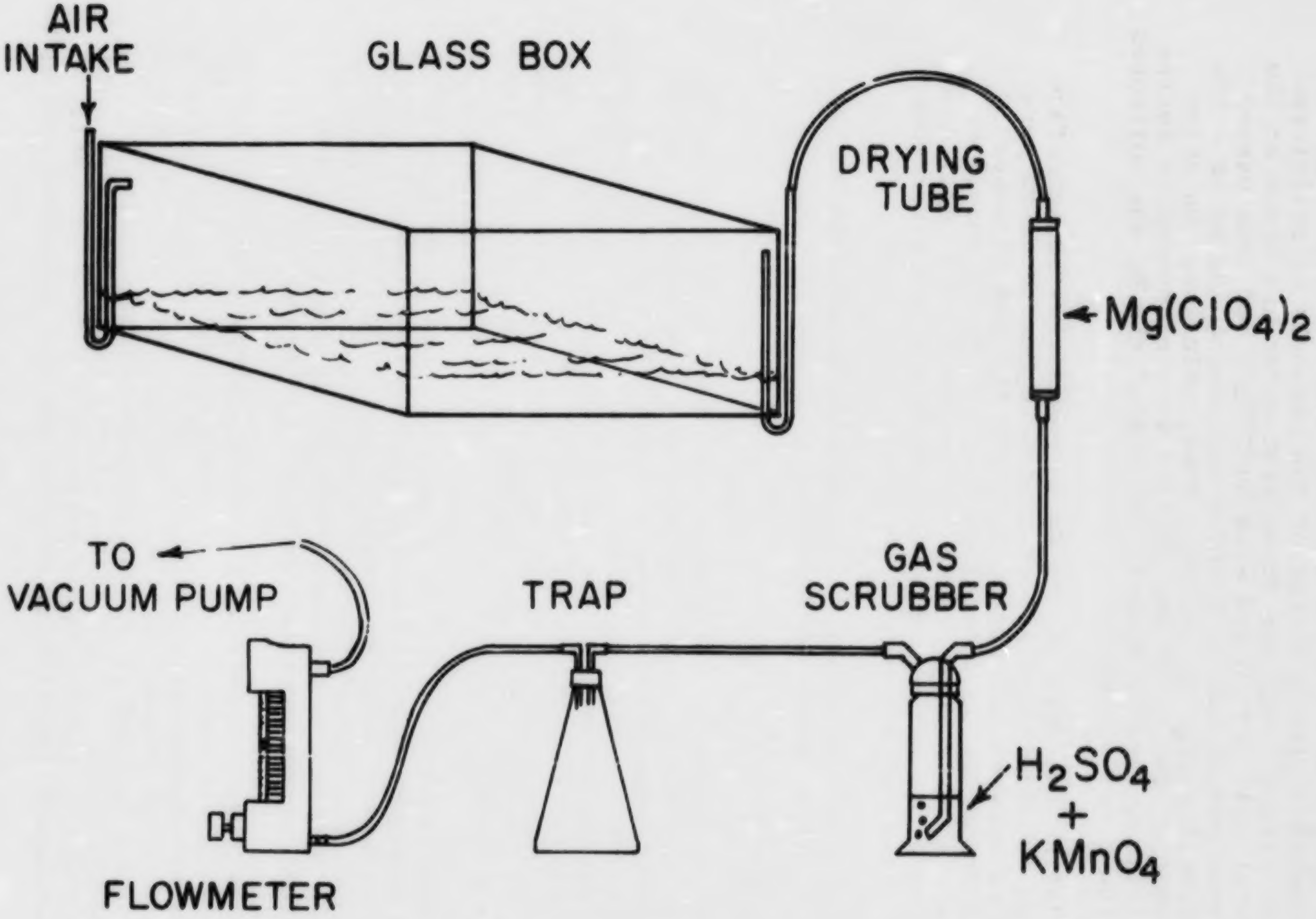
AIR
INTAKE
GLASS BOX
DRYING
TUBE
$Mg(ClO_4)_2$
TO
VACUUM PUMP
TRAP
GAS
SCRUBBER
H_2SO_4
+
$KMnO_4$
FLOWMETER

FIGURE 5. AIR SAMPLING SYSTEM.

and algae poured off from the heavier sand. The sand was rinsed once with tap water and was combined with the first suspension removed. The total volume of the resulting suspension was measured, blended for one minute, and subsampled for Hg analyses, organic matter determinations, and algal counts.

Export

The original plans were such that the organic particulate material leaving the channels would be collected quantitatively by a series of screens and nets of decreasing mesh and analyzed for both Hg and organic content. It became obvious very soon after sampling started that this method would not work because of the almost immediate plugging of the screens by very large amounts of a slime associated with the material moving out of the channels, and another method for collecting export was developed.

In the early stages of the project, virtually all of the material moving downstream was floating. Therefore, two stainless-steel screen skimmers were suspended in each channel, one at the effluent end and one midway between ends. The mats that formed behind the skimmers were harvested quantitatively with a slightly submerged plastic dishpan and transferred to a 4-l graduated cylinder for measurement of total volume. The contents of the graduated cylinder were thoroughly mixed, a 200-ml volume removed and blended for one minute, and two 10-ml subsamples taken, one for organic content determination, one for Hg analysis.

Periphyton

Channel Walls-

Shortly after the water input to channels was started in September, 1971, 40 plastic strips (10 X 20 cm) of the same PVC material lining the channels, were suspended at upstream and downstream locations in each channel. These strips were removed periodically and the periphyton was scraped off into a small amount of water. Biomass determinations and Hg analyses were made on samples of a blended suspension of this material. Sampling of these plastic strips was discontinued in October, 1973, because the large growth of an emergent aquatic plant, Juncus diffusissimus, in the channels interferred with the hanging strips to the extent that they could no longer be considered representative of the channel walls. A method was then devised so that a known area of channel wall could be scraped and the periphyton analyzed as above. A plexiglass box with the top and one side removed was placed tightly against the wall with the top above the water line. The enclosed section of wall, an area 47-mm wide and 125-mm deep, was scraped clean with the flat end of a plastic stirring rod and the slurry was transferred to a beaker by means of a hydrometer suction tube. All samples were taken from the north-facing (shaded) wall. These samples were treated the same as the plastic strip samples except that a 5-ml aliquot was saved for algal enumeration.

Plexiglass Plates-

Plexiglass plates measuring 10 X 10 X 0.63 cm were placed in the channels in December, 1971, to provide substrates for periphytic growth. Four trays carrying a total of 32 vertical plates (the sides were parallel to the direction of stream flow) were placed at the head and tail of each of the six channels. The same number of horizontal plates were placed on the sand substrate interspersed with the racks holding the vertical slides. During the first two years, two plates of each orientation were removed monthly for biomass determination and algal enumeration. In the following year, one vertical plate was removed monthly from each position in each stream for Hg analyses in addition to the algal counts and biomass determinations. The horizontal plates were abandoned after April, 1973, because of the high variability between them (Ferens, 1974). Vacated positions were refilled with new plates that were allowed to colonize for at least six months before being removed. During the last year of the study, all of the plates used had been in the streams for at least one year.

Periphytic growth on the plates was carefully scraped with a plastic stirring rod and washed with a small amount of distilled water into a beaker. This sample was homogenized for one minute in a Waring blender, the total volume was determined, and measured subsamples were removed after additional mixing for Hg analysis, biomass determination, and algal counts.

Between July, 1972, and April, 1973, two clean plates were placed each month at both positions (head and tail) in all channels and harvested after one week of exposure as a study of short-term productivity. These plates were sampled as described above except that the entire suspension was filtered for biomass determinations.

In June of 1974, five months after the mercury inputs had been stopped, 10 clean plates were placed in channel 6 (formerly 5 µg/l). Two plates were harvested biweekly over a three month period and handled as described above.

Glass Slides-

Glass microscope slides (25 X 75 mm) were used to directly study the initial colonization of periphytic algae in the artificial streams. Slides were hand-washed with a mild cleanser, thoroughly rinsed with deionized water, and placed at upstream and downstream locations in each channel on five occasions, two before the Hg inputs were stopped (October 31, 1973 and December 7, 1973) and three after the inputs were stopped (January 30, 1974, April 25, 1974 and October 23, 1974). Eight to ten slides were suspended 7.6 cm above the sand substrate at each location and oriented with their flat sides facing the water flow. One slide was removed from each location in each channel at three-to-seven-day intervals for at least one month, the downstream side was wiped clean of periphyton and the upstream side was counted as described below. On two occasions (after 40 and 80 days of colonization), sets of glass slides were scraped and rinsed with distilled water and the biomass and Hg concentrations were determined.

Macrophytes

The initial project plans had made no provision for sampling macrophytes. By spring of 1973, however, it became evident that a macrophyte, *Juncus diffusissimus*, was to assume increasing importance in the channel communities. Routine sampling of this *Juncus* for Hg analyses was initiated in June, 1973. Individual plants were removed by hand from the channels, the roots were washed free of sand, the leaves were washed free of periphyton and these two portions separated. During the last year of the project, quantitative estimates of the macrophyte standing crop biomasses were made from plants removed with the large core sediment samples previously described. These plants were also used for Hg analyses.

Aquatic Insects

In November, 1971, 16 rectangular pyrex baking dishes (15.7 X 26 X 4 cm) were filled with sand and placed in upstream and downstream locations in each channel. One dish from each position was removed monthly, the organisms being retained by either a rectangular stainless-steel screen box fitted inside the walls of the dish or by a flat piece of screen held against the surface as the dish was removed. The contents of the dishes were transferred to stainless-steel buckets, the dishes washed, filled with sand and replaced in their former positions. Tap water was added to the bucket, the contents stirred by hand and the suspended material poured into white enamel pans from which the living organisms were removed with forceps. The procedure was repeated until nothing was found in two consecutive rinsings. Insects removed were preserved in 70% or 80% ethanol until they could be identified and counted. All dishes sampled had been in the channels approximately one year before removal.

Aquatic insects were qualitatively sampled by sorting through the detritus collected on the fish-retaining screens at the tails of the streams and also by collecting individuals emerging from the streams onto the plants or into the air. Adult odonates laying eggs in the streams were netted and preserved throughout the warmer months to aid in the identification of nymphs.

Fish

From April, 1972, until January, 1973, the fish were collected from the channels with small nets. This method was inefficient because of the care required to prevent disturbance of the wall and bottom communities. A small portable shocker was constructed and utilized for the remainder of the project. Fish were removed from the screen cages with a small dip net.

ANALYTICAL PROCEDURES

General

All analyses were carried out on a Coleman MAS-50 Hg Analyzer System modified by the addition of a digital read-out and fused quartz windows on the absorption cell. Standards were prepared prior to each set of analyses from a 1.0 μg/ml solution made weekly from a commercially available 1000 μg/ml standard (Harleco Corp., Philadelphia, Pa.). Standard curves derived by the "standard additions" method with fish and periphyton digestates did not differ significantly from those derived by adding measured amounts of Hg to 100 mls of a deionized H_2O solution containing 5 ml concentrated H_2SO_4, 1 ml concentration HNO_3, and 1 ml of a 6% (wt/vol) $KMnO_4$ solution. Therefore, the simple acid-permanganate solution was used for the preparation of standards.

Regardless of the material sampled, an acid-permanganate solution resulted from the digestion procedure. This was reduced with a 1.5% (wt/vol) hydroxylamine hydrochloride solution, further reduced with a 10% (wt/vol) $SnCl_2$ in 20% (vol/vol) HCL solution, and the resulting free Hg sparged from solution by a recirculating current of air which passed through a $Mg(ClO_4)_2$ drying tube into the UV absorption cell of the Hg detector (Coleman, 1970). The maximum absorbance reached was recorded. Analytical procedures were checked by analyses made of Hg standards in gelatin (Eastman Kodak Co., Rochester, N.Y.) and by a series of recovery experiments with samples spiked both with inorganic (Harleco) and methylmercury (Alfa Inorganics, Beverly, Mass.) standards. In all cases recoveries ranged from 95-103%.

The few methylmercury analyses that are reported were performed by personnel of the Environmental Protection Agency's Southeast Environmental Research Laboratory under the direction of Dr. Harvey W. Holm using the technique of Longbottom et al. (1972).

Water

Analyses were made of 100-ml aliquots of both filtered (0.3 µm Gelman Type A glass fiber filters) and unfiltered water. These samples were treated with 5 ml of concentrated H_2SO_4, 1 ml of concentrated HNO_3, and 1 ml of a 6% (wt/vol) $KMnO_4$ solution and allowed to stand at least 30 min before being analyzed. Results from this technique were compared against those obtained from a procedure which included the addition of $K_2S_2O_8$ and a heat treatment. These extra steps were found to be unnecessary for the channel water samples although they may be required in some cases (EPA, 1971). Filters tested for Hg were found to contain less than 0.002 µg of this metal. Filtration of 100 ml of 0.1 µg Hg/l solutions of $HgCl_2$ in 1% (vol/vol) HNO_3 showed that the filters removed less than 1% of the dissolved Hg from solution.

Air

The gas scrubbers containing the 100 ml of H_2SO_4-$KMnO_4$ solution used for extracting Hg from the air were carried to the laboratory and the excess permanganate reduced by the addition of crystaline hydroxylamine hydrochloride. The contents of the scrubbers were then transferred to BOD bottles and handled in the usual manner, starting with the addition of $SnCl_2$. In some cases 10-ml aliquots were used instead of the entire sample when high levels were expected, based on previous data. These were diluted to 100 ml with deionized water. Samples (100 ml) of the H_2SO_4-$KMnO_4$

solution used in the sample scrubbers were analyzed so that corrections could be made for the slight amount of Hg in the reagents.

Sediment

The small (14-mm) cores removed in the early part of the project were cut, while frozen, into three parts. The well-defined organic layer, which ranged from 2-5 mm in thickness, was removed and the remaining sand was divided into two approximately equal portions. The three fractions from each core were dried at 60°C overnight, and cooled in a dessicator. Weighed subsamples were transferred to 100-ml volumetric flasks and treated with 5 ml of a 6% (wt/vol) aqueous $KMnO_4$ solution. The flasks were covered with marbles and autoclaved for 15 min at 121°C (EPA, 1974). After the flasks had cooled, approximately 80 ml of deionized water was added. The flasks were allowed to stand for several hours, and then brought to volume with additional deionized water. The contents of the flasks were transferred to BOD bottles for analysis. For the quantification of organic content, weighed subsamples of the dried core fractions were placed in 40-ml Vycor evaporating dishes and ashed for approximately 18 hr at 400°C.

The sampling procedure for benthic insects effectively separated the organic portion of the sediment in the pyrex dishes from the sand. Virtually all of the organic matter was transferred into the enamel pans with the wash water used to remove the insects. This was saved and the total volume measured when sampling was complete. The water was then mixed to suspend settled matter and a 100-ml portion was removed which was blended and subsampled for Hg analysis and the determination of organic content.

Estimates of the organic matter contained in the blended samples were obtained in one of two ways. A 10-ml sample of the homogenate was either filtered through a prepared 0.3 μm Gelman Type A glass fiber filter, or was placed in a prepared 40-ml Vycor evaporating dish. Filters were washed with deionized water, dried at 110°C, fired at 500°C for at least four hours, and dessicated until they were removed for weighing prior to use. The same treatment was applied to Vycor dishes except the dishes were scrubbed with a mild abrasive (Bon-Ami) before being rinsed.

Samples on the filters or in the Vycor evaporating dishes were dried to constant weight at 65°C, cooled and weighed.

They were then ashed at 400°C overnight, cooled and reweighed. The loss between the last two weighings was considered to be an estimate of the ash-free dry weight which was used to convert Hg readings to a relative (μg Hg/g ash-free dry weight) value to permit comparisons between samples.

For Hg analyses, a 5- or 10-ml aliquot of the homogenized sample was transferred with a volumetric pipette to a 100-ml volumetric flask. Five ml of concentrated H_2SO_4, 1 ml of concentrated HNO_3 and 5 ml of 6% (wt/vol) aqueous $KMnO_4$ were added to the flask. In the early part of the study, the flasks were loosely capped and autoclaved for 15 min at 121°C; during the remainder of the study, overnight digestion of the sample at room temperature replaced the autoclaving step because studies showed comparable recoveries by both methods. After the digestion process, volumes in the flasks were adjusted to 100 ml with deionized water and the resulting solution transferred to BOD bottles for analysis.

Sand samples from the pyrex dishes were dried at 65°C and weighed amounts (0.5-1.0 g) were transferred to 100-ml volumetric flasks for digestion in the manner described above.

Sediment samples taken with the 5.5-cm coring device during the last part of the study were handled in the same manner as the sediment removed from the pyrex benthic dishes.

Export

Export samples consisted of relatively dense suspensions of periphyton which had sloughed off and floated downstream. When large volumes were involved, as in the case of skimmer samples, these volumes were subsampled. Export samples, as taken from the fish-retaining screens, usually had a total volume of 100 ml or less and the entire sample was used.

The export samples (or measured aliquots of the large samples) were blended for 1 min to homogeneously distribute the suspended material. Five ml of the homogenate were added to 100-ml volumetric flasks and then subjected to the procedure described for the processing of the organic portion of the sediments.

The organic content of the export homogenates was determined by the method described for the organic portion of the sediments. Comparisons made between estimates derived from filter data and Vycor dish data showed that the Vycor dish results were consistently higher by about 5%. This indicates that the blending process may release soluble organic cell constituents which pass through the filter.

The relationship between wet weight and ash-free dry weight was determined on three occasions. Composite samples of export material were collected, blended, centrifuged and weighed amounts of the resulting mass placed in Vycor dishes for drying and ashing. Data show that the weight loss on ignition was (5.9%) of the wet weight which agrees well with the value of 6.4% calculated from data presented by Sladecek and Sladeckova (1963) for periphyton colonizing vertical glass slides.

Periphyton

All periphyton sampling procedures resulted in aqueous suspensions of the material scraped from the various substrates. These suspensions were blended for 1 min and treated in the same manner as described for the organic portion of the sediments except that an additional subsample was taken for algal counts. The volume taken for digestion was a function of the density of the suspended matter in the sample.

Macrophytes

The washed leaves and roots of the Juncus plants removed from the channels were allowed to dry at room temperature for 24-48 hr. The roots and stems were cut into small pieces and weighed portions placed in 100-ml volumetric flasks and treated with 5 ml of concentrated H_2SO_4 and 1 ml of concentrated HNO_3. The stoppered flasks were manipulated so that all the plant material was in contact with the acid, and then allowed to stand for 18-20 hr. Five ml of 6% $KMnO_4$ was then added and the flasks stoppered after the initial frothing had stopped. The samples were allowed to digest at room temperature for an additional
After this period, the volumes were adjusted to 100 ml and the contents of the flask transferred to BOD bottles for analysis. This procedure was sufficient to dissolve and oxidize the plant tissues and no residues were visible when the permanganate coloration was destroyed with hydroxylamine hydrochloride during the analytical process.

Weighed and dried samples of roots and stems were oven dried at 60°C for 18-20 hr, cooled in a dessicator, weighed, ashed at 400°C, cooled and weighed again. The weight lost during the ashing procedure was used to calculate the percent organic matter in the original air dried sample. The figure was then used to calculate the weight of the organic component of the plant samples analyzed for Hg.

Insects

Insects collected from the screens at the ends of the channels were blotted dry, weighed, and placed in 100-ml volumetric flasks. Five ml of concentrated H_2SO_4 and 1 ml of concentrated HNO_3 were added to the flasks which were allowed to stand, with occasional shaking, until a clear solution resulted. Five ml of a 6% (wt/vol) $KMnO_4$ solution were then added to each flask. The flasks were stoppered after initial frothing had ceased and allowed to stand for at least 16 hr at room temperature. They were then filled to 100 ml with deionized water and transferred to BOD bottles for analysis.

Fish

Mosquitofish were killed by submersion in hot water, blotted dry, weighed and placed in 100-ml volumetric flasks. They were then processed in the same manner as described for insects. When the fish were large (> 0.5 g), twice the usual amount of acids and $KMnO_4$ were added. Reagent blanks were prepared in the same manner.

ALGAL COUNTS

Representative subsamples (approximately 5 ml) of the homogenates resulting from the processing of sediment cores, channel walls and plexiglass plates were placed in 15 X 125-mm tubes and refrigerated in the dark until counts could be made. Usually samples were counted the same day they were collected, however, a few were stored for as long as two days.

For counting, the samples were brought to room temperature, resuspended by shaking the test tube at high speed with a vortex type mixer (Cole-Palmer Super-Mixer) and a 50-µl portion removed immediately with an Oxford micropipettor fitted with a disposable tip. The portion was placed as a drop on a clean microscope slide and spread evenly under a 22 X 22-mm, #1 glass coverslip. The algal cells observed in measured areas of the slide were identified and counted at 400 X with a Nikon Model SKe phase-contrast microscope. An attempt was made to identify and count a total of 200 cells from each slide although up to 1000 cells were counted on very dense slides. On a few occasions, because of the low numbers of organisms in the samples, counting was terminated at 100 cells. During the last part of the study, a replicate count from another measured drop was made for each sample.

All cells in colonies and filaments were counted individually. Vegetative cells were counted if chloroplasts were present, and tiny, highly motile flagellates were not counted unless they were observed in resting stages.

Qualitative observations of blended and unblended periphytic material indicated only one common algal species was altered beyond recognition in the blending process (Eremosphaeria viridis) while filamentous forms were commonly broken up into single cells or small groups of cells permitting more accurate enumeration.

Algal counts for individual species were converted to relative values (cells/mm^2 of substrate) for treatment comparisons. To compare total populations, the volume of each species was estimated from average measurements of the cells (APHA, 1965) and the total cell volumes (in cubic standard units, 1 CSU = 8000 mm^3) per unit area (mm^2) of substrate compared between treatments. The total organic content of periphyton attached to each substrate was determined from dryed and ashed subsamples of the same suspension on which the algal counts were made. Diversity and evenness values were calculated from equations given by Pielou (1969) for subsamples from large populations,

$$\overline{H}=-\sum_{i=1}^{S} (n_i/N) \log_2 (n_i/N) \text{ , and}$$

$$E=\overline{H}/\log_2 S$$

where S is the number of species, N is the total number of cells counted, n_i is the number of cells in the i^{th} species, $\overline{H}$ is the Shannon Weaver diversity and E is the evenness.

Algae colonizing the glass microscope slides suspended in the channels were counted directly. After a slide was removed, the downstream surface was wiped clean and a coverslip was placed on that end of the upstream surface that had been deepest in the water. Again, a known area was examined and the algal species identified and counted.

STATISTICAL TREATMENT

The amount of replication that was possible in sampling the channel components was limited by the physical size of the channels, the long duration of the study, and the amount of personnel time available. At some time during the project period, however, at least one estimate was made of the variability associated with each method and these variability data have been presented in the appropriate section.

In analyzing the large quantity of routine data collected, the positional effects resulting from the difference between upstream and downstream stations and the differences between channels treated alike have been largely ignored. Separation of the effects of the most important factors influencing the results, Hg level and time, were made by a two-way analysis of variance (ANOVA, fixed effects model) by means of subroutines included in the Statistical Analysis System (SAS) (Service, 1972). Correlations, regressions, and basic calculations were done either with SAS subroutines or with BASIC programs specifically written for the Nova 1200 computer associated with the stream facility.

RELATED STUDIES

General

During the project period, several short-term studies relevant to the stream research were carried out. Some of these were initiated to confirm channel results, others were done to provide information not available from the channels. The rationale for these short-term studies and the methods used will be presented in the following paragraphs. The results and conclusions will be discussed in Section VII.

Bacterial Counts

Since the sensitivity of microorganisms to Hg salts is well known, the initial project plans included daily sampling of water for total bacterial counts. From the beginning of June, 1972, until the middle of October, 1972, samples of water were taken between noon and 2 PM daily in sterile dilution bottles from head and tail positions in each channel. The suspended bacteria were counted by serial dilution pour plates made with tryptone glucose extract agar. Because no differences were found between treatments, sampling was reduced to a weekly schedule in November, 1972, and discontinued completely at the end of the year. The Hg levels in the channels were below those found to have an effect on heterotrophic bacteria by Albright et al. (1972).

Productivity Estimates

The original project also included plans for channel productivity determinations based on upstream-downstream measurements of dissolved oxygen (Odum, 1956) and pH (Beyers, 1970). For this purpose, Schneider Instrument Co. RM-15 water quality monitors were borrowed from the EPA's Southeast Environmental Research Laboratory (SERL) Athens, Georgia, and installed in small buildings located at both ends of each channel and a system constructed which switched the water input to the monitors from one channel to another every 10 min. Dissolved oxygen, conductivity, pH, and temperature were recorded by both monitors and the monitor near the head of the channels also recorded solar input. Plans to interface the monitors with a digital data-processing system based on a Nova 1200 computer were abandoned when it became apparent from the analog outputs that there were no measurable differences, except with respect to temperature, between the ends of the channels. The water was generally saturated with oxygen at all times. The maintenance and calibration of the monitors was extremely time consuming and the fulltime monitoring of water quality was discontinued.

During the period the monitors were in use, the standing crop biomasses in the channels were relatively small. To see if the lack of usable results was simply a result of the lack of biota, upstream-downstream measurements of oxygen and pH were made on several occasions shortly before the termination of the project. There were well-defined differences between stations and it appears that productivity measurements could be made in the channels after sufficient growth has occurred.

Diurnal Uptake Patterns

There was, and still is, some question as to whether or not the removal of Hg from water is mediated by the physiological activities of organisms in aquatic systems. In June and August of 1972, 25 mci of Hg-197 was input over a 24-hr period into the channels maintained at 1.0 µg Hg/l (2 and 5). The low-level channels set at 0.01 µg/l could not be used because the specific activity of commercially available Hg-197 was too low. During the 24-hr input period, and for several hours afterwards, 5-ml water samples (unfiltered) were taken hourly from three locations in both channels, from the effluent stream below the shredded rubber Hg removal systems, and from a small pond connecting with the effluent channels.

All samples were counted in a deep-well, 5-cm NaI (Tl) scintillation crystal coupled to a 400-channel pulse height analyser. Counting times were adjusted to maintain a relative counting error of approximately 10%. The purpose of taking the hourly water samples was to look for diurnal patterns of Hg uptake by the channel communities as determined by differences in activity between upstream and downstream samples of the same water mass.

An intensive 26-hr survey was also done in January, 1974, when all six channels were sampled for water at upstream and downstream stations, and for Hg losses at the air-water interface at the mid-point of the channels every 2 hr. Both filtered and unfiltered water subsamples were analyzed for Hg by the methods described previously.

Continuous-flow Chambers

To determine if the responses to Hg observed in the channels could be verified under conditions of reduced interspecific interactions, continuous-flow chambers (CFC) were designed and built to test Hg uptake and growth responses of specific channel organisms in a more-controlled fashion. The system is illustrated in Figure 6. Water from the well feeding the artificial streams was passed through a limestone treatment similar to the system used for the channels and passed through the plexiglass chambers to give a velocity equal to that used in the channels (0.013 m/sec.) Mercuric chloride was pumped into the input line of three of the plexiglass chambers to provide a concentration of 5 µg Hg/l and the other three chambers served as controls.

The CFC system was first used to determine direct uptake rates of Hg from water by mosquitofish and by a corixid hemipteran (Hespercorixa sp.). For these uptake studies, 30 animals were placed in each chamber and 5 were removed at each sampling time.

Attempts were made to isolate the dominant algal species of the artificial streams to use in the CFC system. Suspensions of channel periphyton were sprayed with a spray-diffuser (Pringsheim, 1946) onto agar plates of Bold's medium (Bischoff and Bold, 1963), modified by the elimination of buffers and the addition of vitamins, or onto agar plates of "Algal-Grow," a product of Carolina Biological Supply Co., Burlington, N. C. Five species were isolated and maintained on agar slants. Transfers to aqueous media were made to develop seeding stocks for the CFC system.

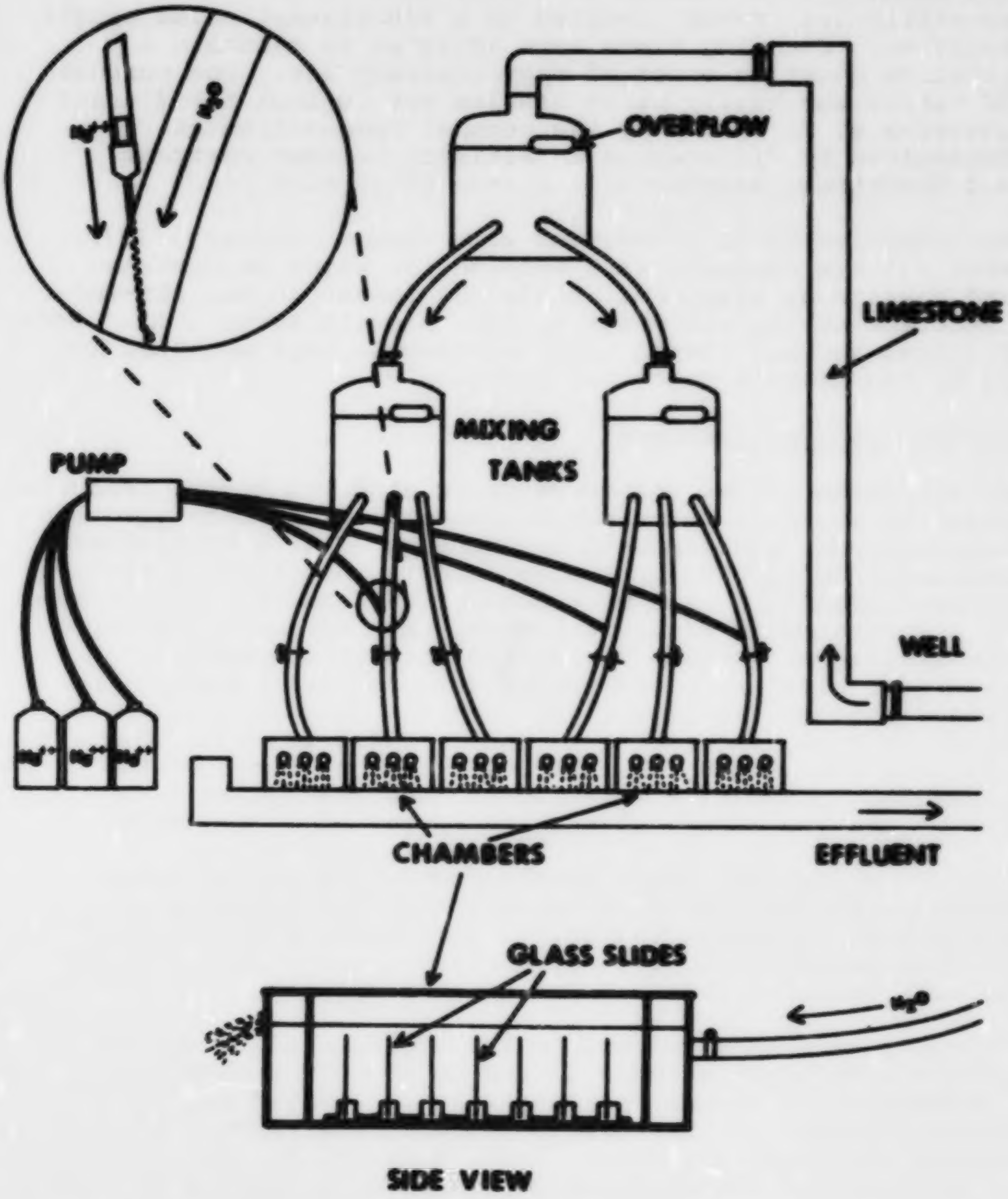
Hg^{++}
H_2O
OVERFLOW
LIMESTONE
MIXING TANKS
PUMP
Hg^{++}
Hg^{++}
Hg^{++}
WELL
CHAMBERS
EFFLUENT
GLASS SLIDES
H_2O
SIDE VIEW

FIGURE 6. CONTINUOUS FLOW CHAMBER SYSTEM

For the study of the effects of Hg on the algal species, racks holding glass microscope slides perpendicular to the direction of flow were installed in the chambers. Mixed or unialgal cultures were poured into the two mixing tanks. The colonization of the glass slides was observed, by the methods previously described for the channel glass slides, for a period of up to 27 days.

Biological Half-life of Mercury in Mosquitofish

After the Hg inputs to the channels were stopped, 200 Hg-contaminated mosquitofish were collected from the effluent system of the channels. These fish were placed in 200-l tanks, 40 per tank, into which a constant flow of fresh water was piped. The fish were fed with a commercial fish food preparation (Bi-O-Rell) low in Hg (0.20 μg/g dry wt.). Samples of 10 fish were removed periodically and analyzed by the methods previously described. The study was done to compare the rates of Hg loss from contaminated fish transferred to clean systems with those calculated from fish remaining in contaminated systems (the stream channels). It was also done to compare estimates of the biological half-life of Hg in mosquitofish exposed over a long period with estimates based on fish exposed for a short period to relatively high levels (Huckabee and Blaylock, 1973).

SECTION VI

RESULTS AND DISCUSSION

FATE OF MERCURY

General

There are several common ways of expressing analytical results and problems can arise when units are not explicitly stated (Olley, 1973). In the discussions that follow, Hg concentrations in fish and insects are expressed on a wet-weight basis. The concentrations in all other community components, with the exception of the sand portion of the sediment, are based on an ash-free dry weight. The ash free dry weight is the weight lost during the ashing process previously described, and is considered to be an estimate of the total organic content of the sample. Results of analysis made on the sand component of the sediment are based on an oven-dry weight, the usual method for expressing sediment results.

Analytical results of export, periphyton, and organic sediment samples, can be converted to a wet-weight basis by multiplying the ash-free dry weight value by 0.06. This conversion is necessary for making comparisons between trophic levels.

Water

During the first 12 months of the project, the average Hg levels in the channels, as calculated from the daily flow and Hg input records, were 1.04 and 1.05 μg/l in channels 2 and 5 respectively, and 0.01 μg/l in channels 3 and 6. Analyses of unfiltered water samples taken approximately 10 m from the Hg input tubes in channels 2 and 5 support the calculated values for these channels.

A summary of Hg analyses of unfiltered water samples collected between August 1, 1973 and January 29, 1974, is presented in Figure 7. The data show that, during this period, the input levels were slightly higher than desired and also that the Hg concentrations in the water leaving the channels were lower than those at the head end. The average uptake in the 1.0-μg/l channels (2 and 5) was 17.5% and in the 5.0-μg/l channels (3 and 6) was 16.8%. The similarity in the relative uptake values at the two different doses indicates there is no saturation of adsorption sites

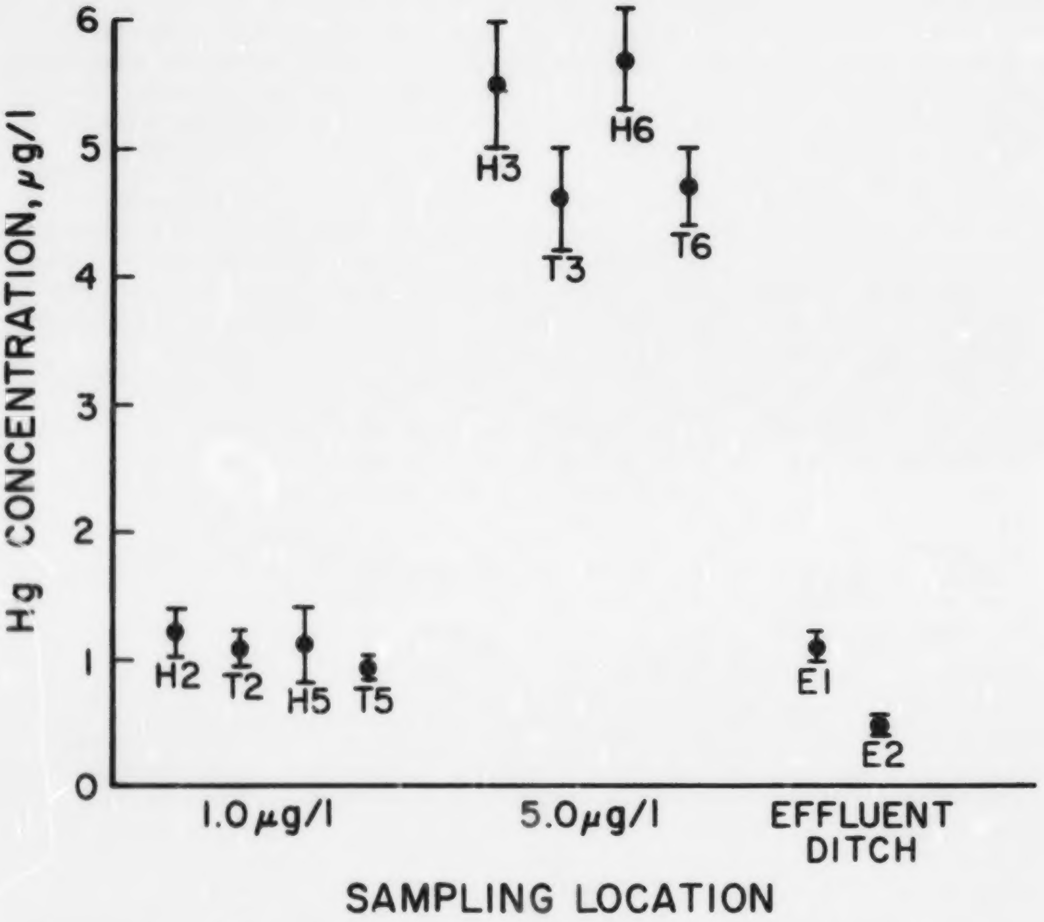

FIGURE 7. TOTAL MERCURY LEVELS ($\bar{X} \pm 2SE$) IN CHANNEL AND EFFLUENT WATER. SAMPLES WERE TAKEN FROM UPSTREAM (H) AND DOWNSTREAM (T) POSITIONS IN EACH CHANNEL AND FROM TWO POSITIONS (E1, E2) IN THE EFFLUENT DITCH DRAINING THE CHANNEL SYSTEM.

at the levels of Hg introduced. The relative uptake values are much lower than those reported by Huckabee and Blaylock (1973) who worked with a 100-m length of natural stream and found that 75% of Hg added as $Hg(NO_3)_2$ was removed. The natural stream Huckabee and Blaylock (1973) studied was much more turbulent than the artificial streams, and probably supported a higher total biomass per unit area.

That turbulence might affect the uptake of Hg is shown by the data presented in Figure 7 for the two positions in the effluent stream. Although the distance between sampling stations in this stream was about the same as between the two channel stations, there was a greater relative uptake of Hg (54.6%) from the effluent water, even though the retention time of the water in this section was short. The greater uptake might be explained by the high turbulence in this region resulting in greater probability of contact between a Hg atom and some portion of the stream wall. The biological communities and lining (poured concrete) in the effluent region, of course, were quite different from those in the experimental channels and the possibility of differential uptakes resulting from differences in community structure and channel lining cannot be disregarded.

The results of the two Hg-197 diurnal uptake studies are summarized in Figures 8 and 9. No uptake patterns are evident, although the variability of the data is high, probably due to the inclusion of particulates in the sample. In the June, 1972, experiment, an average of 12.5% of the radiomercury was removed from the water. In August, 1972, this value increased to 17.9%, probably as a result of the increased biomass in the channels.

The results of the 26-hr intensive water sampling done in January, 1974, are presented in Figure 10. The data show that an average of 13.2% of the Hg added was retained in the 1.0-µg/l channels and 15.6% in the 5.0-µg/l channels. The bimodal uptake curve in both treatment levels is difficult to relate to the biological activity in the channels. A diurnal curve of the type described by Odum (1956) for dissolved oxygen levels or by Beyers (1963, 1965) for carbon dioxide uptake and release in aquatic systems, with a single maximum and minimum each 24-hr period, would be expected if Hg uptake were related to the diurnal photosynthetic or respiratory patterns of the channel communities.

All data pertaining to uptake (Figures 7-10) show that a constant proportion of the added Hg was removed regardless

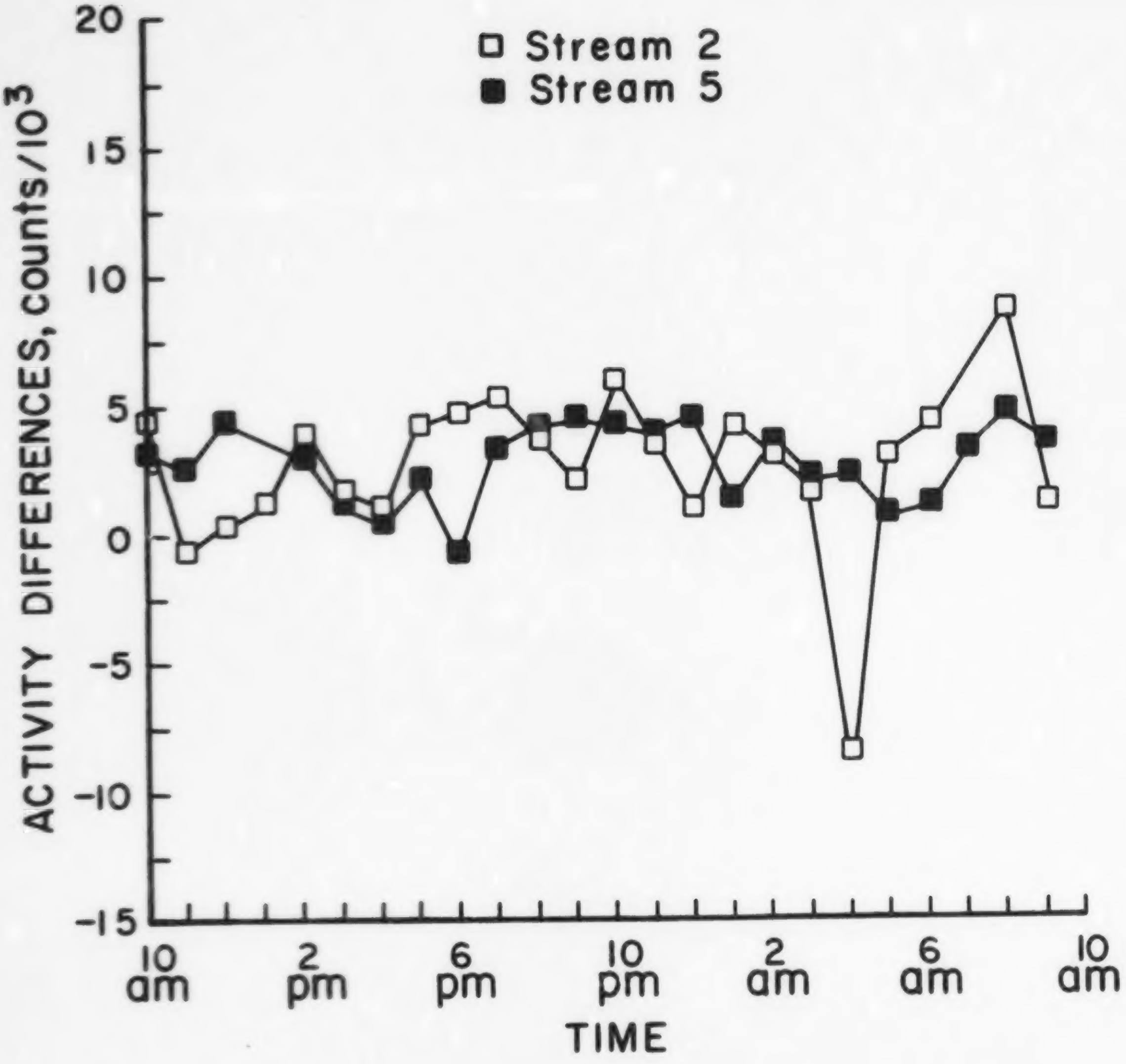

Figure 8. Diurnal radiomercury uptake curve, June, 1972. Plotted are the differences between radiation levels in upstream water samples taken at the indicated time and downstream samples taken two hours later.

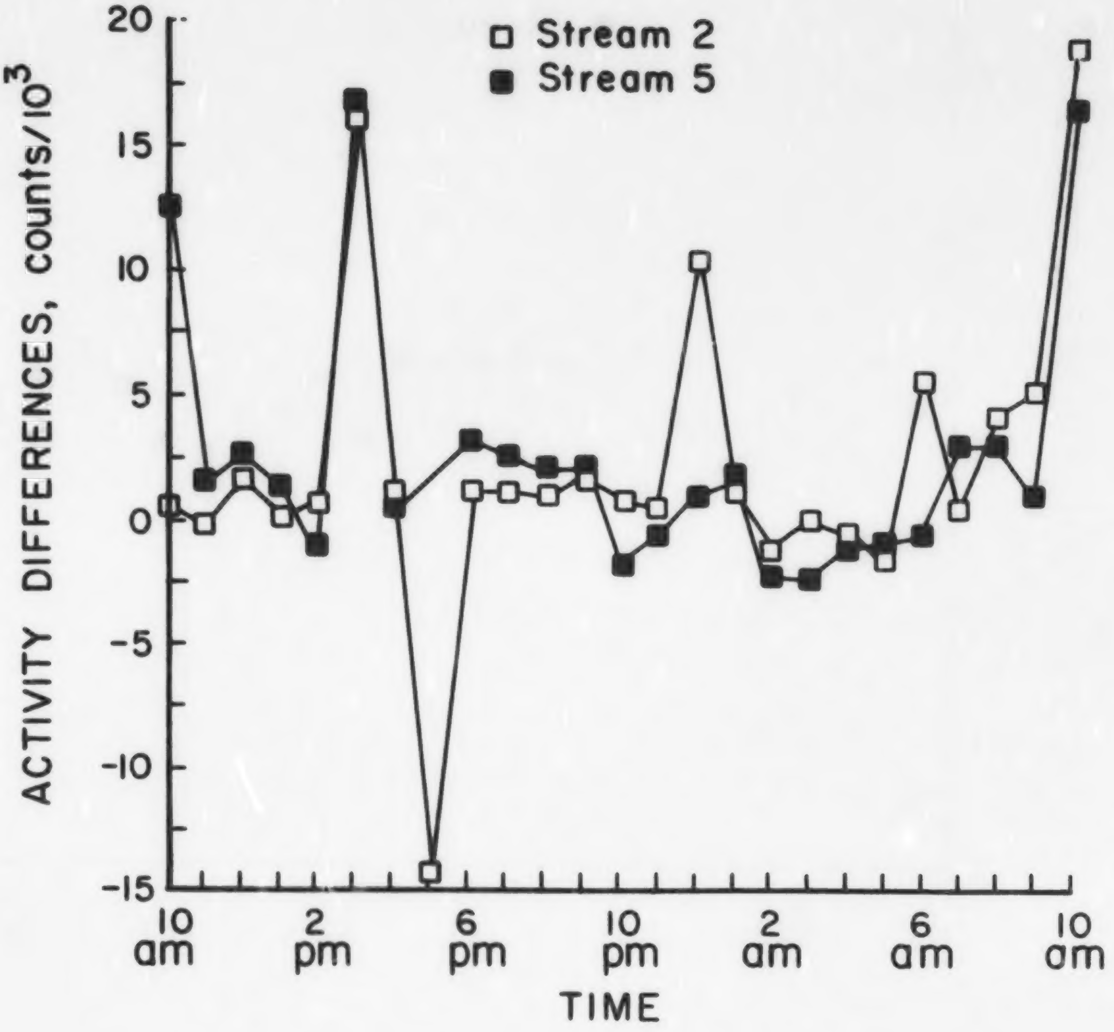

FIGURE 9. DIURNAL RADIOMERCURY UPTAKE CURVE, AUGUST, 1972. PLOTTED ARE THE DIFFERENCES BETWEEN RADIATION LEVELS IN UPSTREAM WATER SAMPLES TAKEN AT THE INDICATED TIME AND DOWNSTREAM SAMPLES TAKEN TWO HOURS LATER.

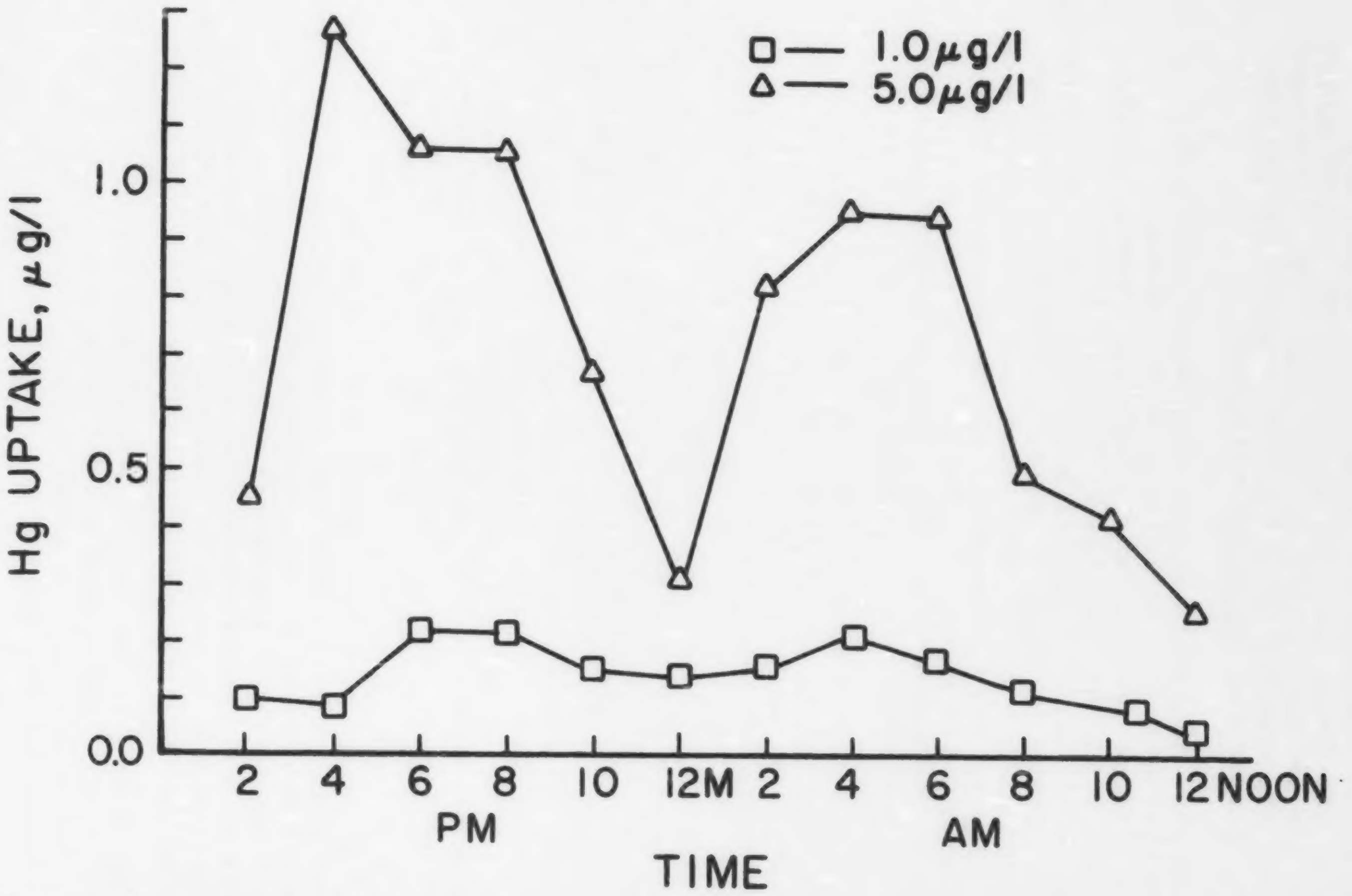

FIGURE 10. DIURNAL MERCURY UPTAKE IN TREATED CHANNELS ON JANUARY 22-23, 1974. PLOTTED ARE THE DIFFERENCES BETWEEN HG LEVELS IN UNFILTERED UPSTREAM SAMPLES TAKEN AT THE INDICATED TIME AND DOWNSTREAM SAMPLES TAKEN TWO HOURS LATER.

of dose, which indicates there was no saturation of potential binding sites at Hg levels up to 5.0 μg/l. These results differ from those reported for Chlorella by Matsui and Gloyna (1972) who found that the higher the Hg concentration, the greater the percent uptake by this alga. The difference may be due to the complex nature of the channel communities compared to unispecific Chlorella cultures.

Figures 8-10 show no clear pattern that would couple Hg uptake with the metabolic activities of the channel communities. These results, which indicate passive uptake, are consistent with the findings of Hannerz (1968), Glooschenko (1969) and Fujita and Hashizume (1972) who attribute inorganic Hg uptake in algae to surface adsorption although Hannan and Patouillet (1972) concluded that in the freshwater algae they studied, Hg uptake could not be accounted for by surface adsorption alone. Burkett (1975) also found indications of processes other than passive adsorption in the uptake of methylmercury by Cladophora, a green alga.

Data showing the fraction of the Hg in the water that was attached to particulate material in the January, 1974, intensive sampling are presented in Figure 11. In all treated channels, the proportion of the total Hg removed by filtration was greater at the downstream stations than at the upstream stations. The percent of the total Hg bound to particles was greater in the 1.0-μg/l channels than in the 5.0-μg/l channels. This indicates a saturation phenomenon with respect to the suspended particulates which may be related to the number of bacteria in the channel water. Figure 12 summarizes bacterial count data based on results of daily samples taken between June 1, 1972, and October 10, 1972. Counts made after October 10, 1972 fall in the limits presented in Figure 12, even after the dose increase of August 1, 1973. The constant number of bacteria at a given point in all channels provide a fixed number of attachment sites for Hg. If the number of sites were limited, there would be a decreased relative uptake at higher Hg levels. This is what was observed (Figure 12) and, although the results are by no means conclusive, the relationship between the increased number of bacteria at the tail end of the channels and the greater proportion of particulate-bound Hg at these stations strongly implicates the suspended bacteria as adsorbing agents.

After the Hg inputs to the channels were terminated (9 AM, January 29, 1974), water samples were taken hourly for 6 hr at the two sample stations in each channel, and on several occasions during the following month. The water levels

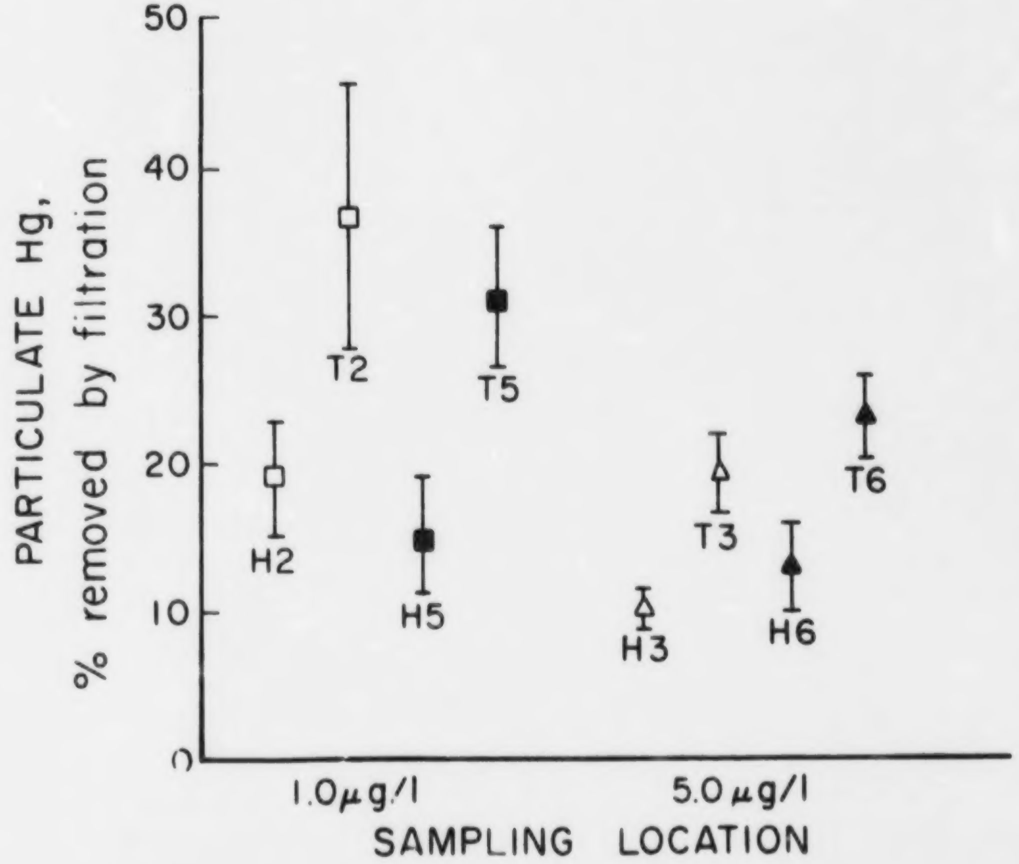

FIGURE 11. PROPORTION OF HG ASSOCIATED WITH PARTICULATES IN WATER SAMPLES. PLOTTED ARE MEANS (± 2SE) BASED ON THE 13 SETS OF SAMPLES TAKEN DURING THE JANUARY, 1974, INTENSIVE SAMPLING PERIOD.

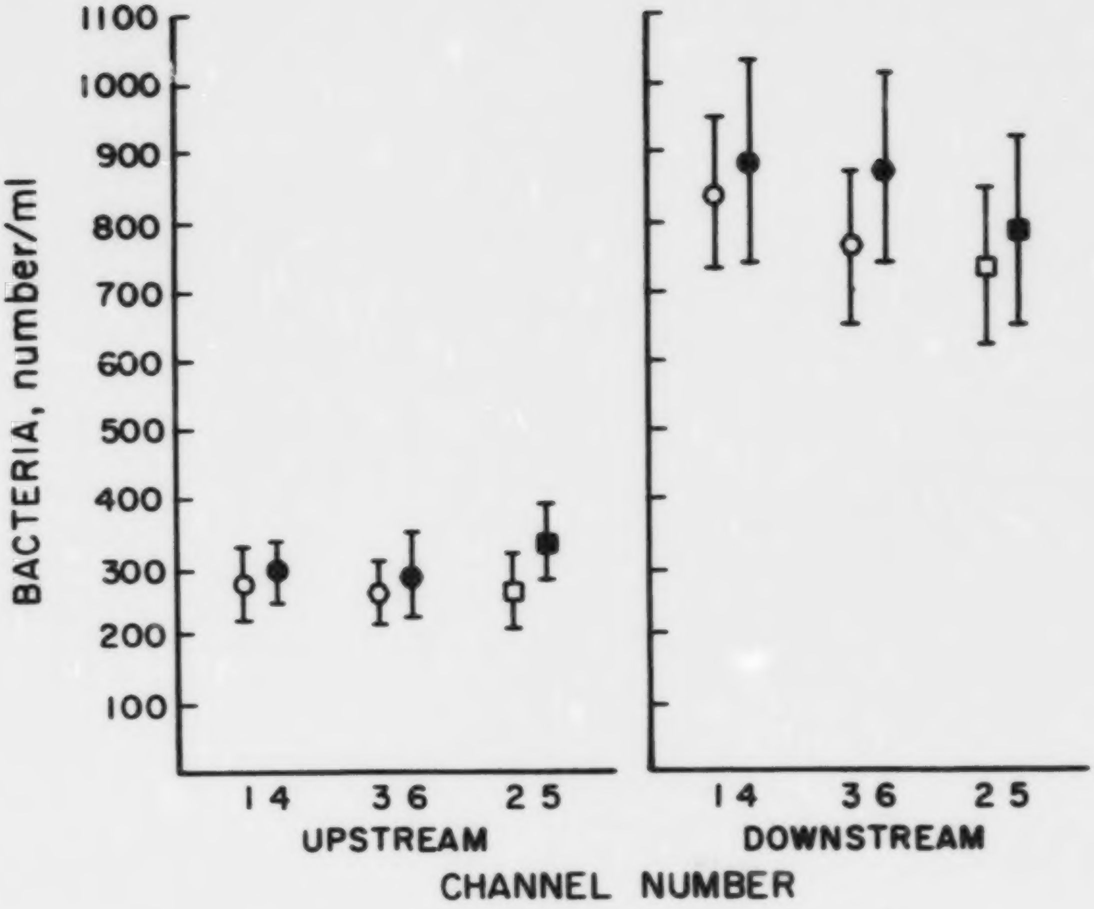

Figure 12. Bacterial populations in channel water. Results ($\bar{x}$ ± 2SE) are based on 130 daily counts made from June to October of 1974.

decreased rapidly and were approximately 0.2 μg/l (filtered samples) in all treated channels by January 31, 1974. By the end of February, 1974, all samples were below the 0.05 μg/l detection limit of the procedure used, even though the Hg levels in the channel periphyton and sediment remained high.

Air

Quantitative data for Hg losses from the channels at the air-water interface were not obtained until December, 1973. No estimates exist, therefore, for losses from the two channels that were receiving an Hg input of 0.01 μg/l.

The air-loss data, which are summarized in Figure 13, show that the releases were highly variable, although related to water concentration, and continued after the Hg inputs were stopped. No losses were measured from the control channels. There appears to be no logical explanation as to why the losses from channel 5 were consistently low, or why the losses decreased rapidly before the Hg inputs were stopped. The data are insufficient to show any seasonal patterns.

The form of Hg lost at the air-water interface is mostly elemental Hg. The results from two air sampling boxes located next to each other in the same channel, one with a silver trap in the line, showed that the filter removed approximately 97% of the Hg before it reached the gas scrubbing system. According to Braman and Johnson (1973), silver does not adsorb dimethyl mercury, and will only partially retain monomethyl mercury compounds, but will quantitatively retain elemental Hg. The specific filters used in this study were checked by inserting them in the line of the Coleman MAS 50 leading into the absorption cell, and then processing a 1-μg Hg standard. No increase in absorption was noted indicating that none of the elemental Hg released in the analytical process passed through the filter. The loss of elemental Hg from aquatic systems has been found in other studies (Fagerstrom and Jernelov, 1972; Spangler et al., 1973; Parks, 1973; Holm and Cox, 1974) and some of the possible mechanisms of its formation have been investigated (Toribara et al., 1970; Alberts et al., 1974; Wood, 1974; Bisogni and Lawrence, 1975).

During the air sampling periods, it appeared that the amount of Hg collected in the traps was a function of the number of bubbles rising from the bottom. Samples of the gas rising from undisturbed sediments were collected and immediately injected into the closed air system of the Hg

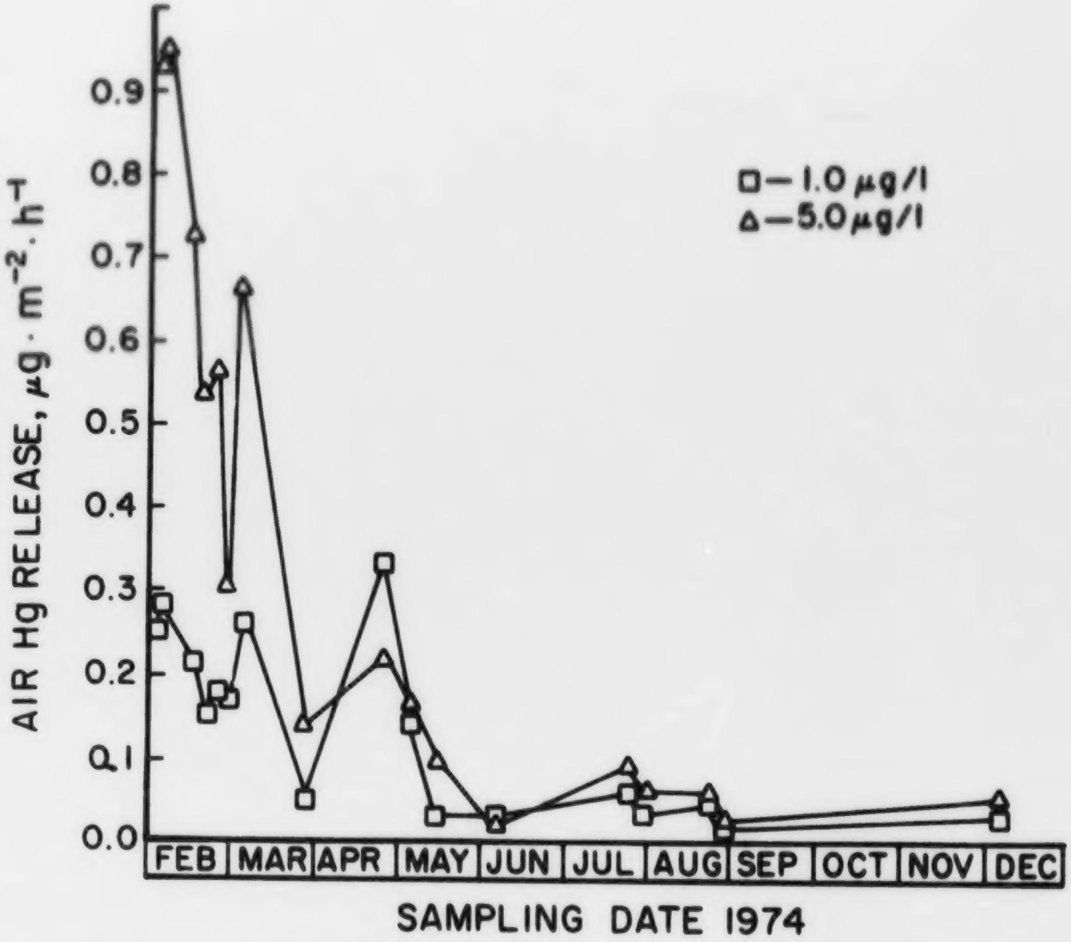

FIGURE 13. AVERAGE MERCURY RELEASES AT THE AIR-WATER INTERFACE OF THE TREATED CHANNELS.

analyzer. This resulted in a significant absorption of the UV beam, and, although no attempts were made to quantify the data, it confirmed the presence of elemental Hg in the gas bubbles. Samples of gas from the control channels were checked and the results were negative. The loss of volatile Hg species with bubbles released from sediments has been suggested as a Hg release mechanism by Bothner and Carpenter (1974).

There were no significant correlations between the air losses and either the organic contents or the Hg content of the sediments under the sampling boxes. Perhaps a correlation would have been observed if some quantification of the living portion of the sediment, especially the bacterial components, could have been made at the same time the air samples were taken. Bacteria are known to be able to release elemental Hg from both inorganic and organic compounds of this metal (Suzuki et al., 1968; Matsumura et al., 1972; Furukawa and Tonomura, 1972), although elemental Hg production can also occur in the absence of bacteria (Alberts et al., 1974; Bisogni and Lawrence, 1975).

Results of the intensive 24-hr air sampling program of January 22-23, 1974, are shown in Figures 14 and 15. The data again show that the releases were greater from the high-level channels than from the low-level channels. The rates of the releases appear to be related to the water concentrations (5:1) rather than to the Hg levels in the sediment on that date (1.6:1, Figure 17). This implies that the reaction-producing elemental Hg was occurring at the water-sediment interface rather than deeper in the sediments.

The air release data shown in Figures 14 and 15 indicate that there may be a diurnal release pattern with a maximum occurring in the late afternoon. This diurnal pattern would not be reflected in the water concentrations of Hg because the maximum air losses observed, if extrapolated to the entire channel, were always less than 1% of the total Hg in the water.

Sediments

When the water inputs to the channels were started in September, 1971, the bottom substrate consisted of a 10-cm layer of washed builders sand. As time passed, a thin mat community consisting of detritus, fungi, bacteria, algae and small invertebrates formed on the sand. The

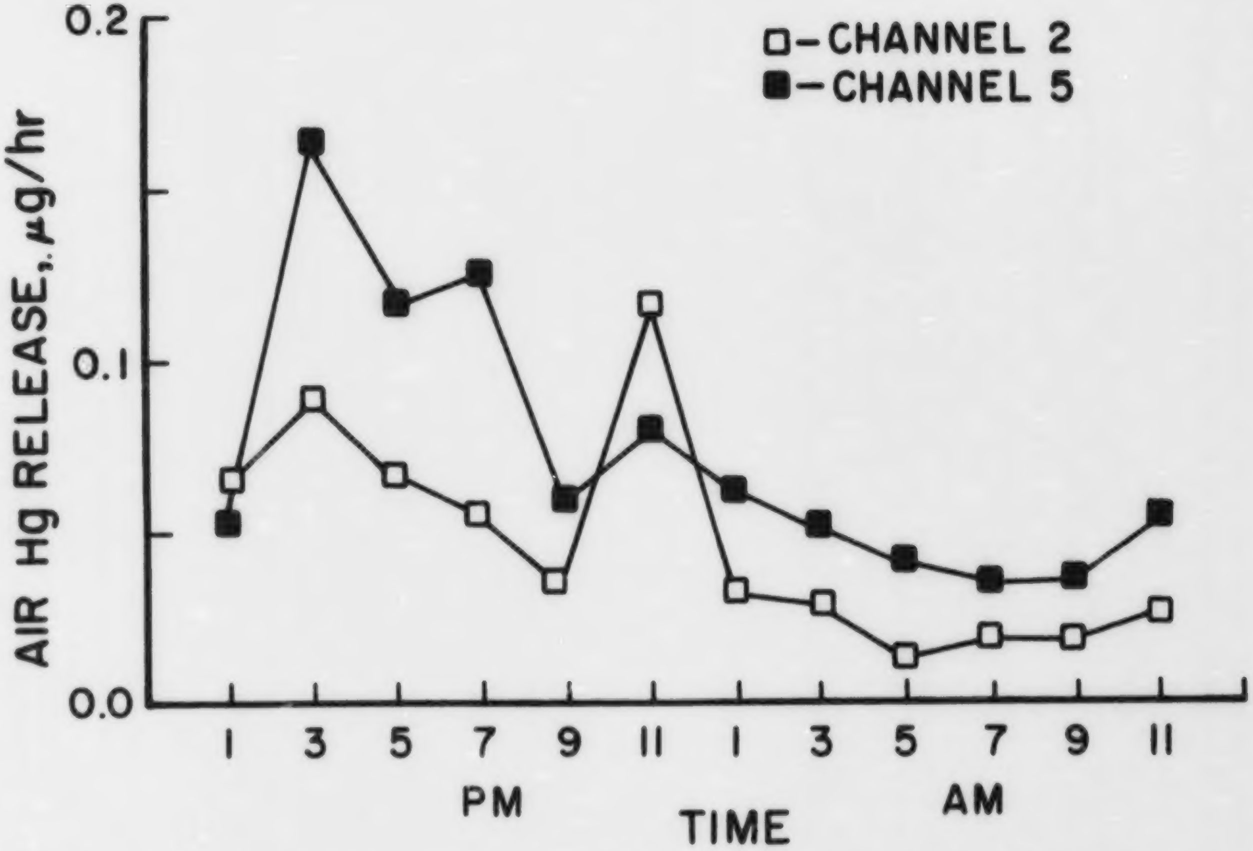

FIGURE 14. DIURNAL MERCURY RELEASES ON JANUARY 22-23, 1975, AT THE AIR-WATER INTERFACE OF CHANNELS RECEIVING AN INPUT OF 1.0 µg/l. THE AREAS SAMPLED WERE 0.186 m^2.

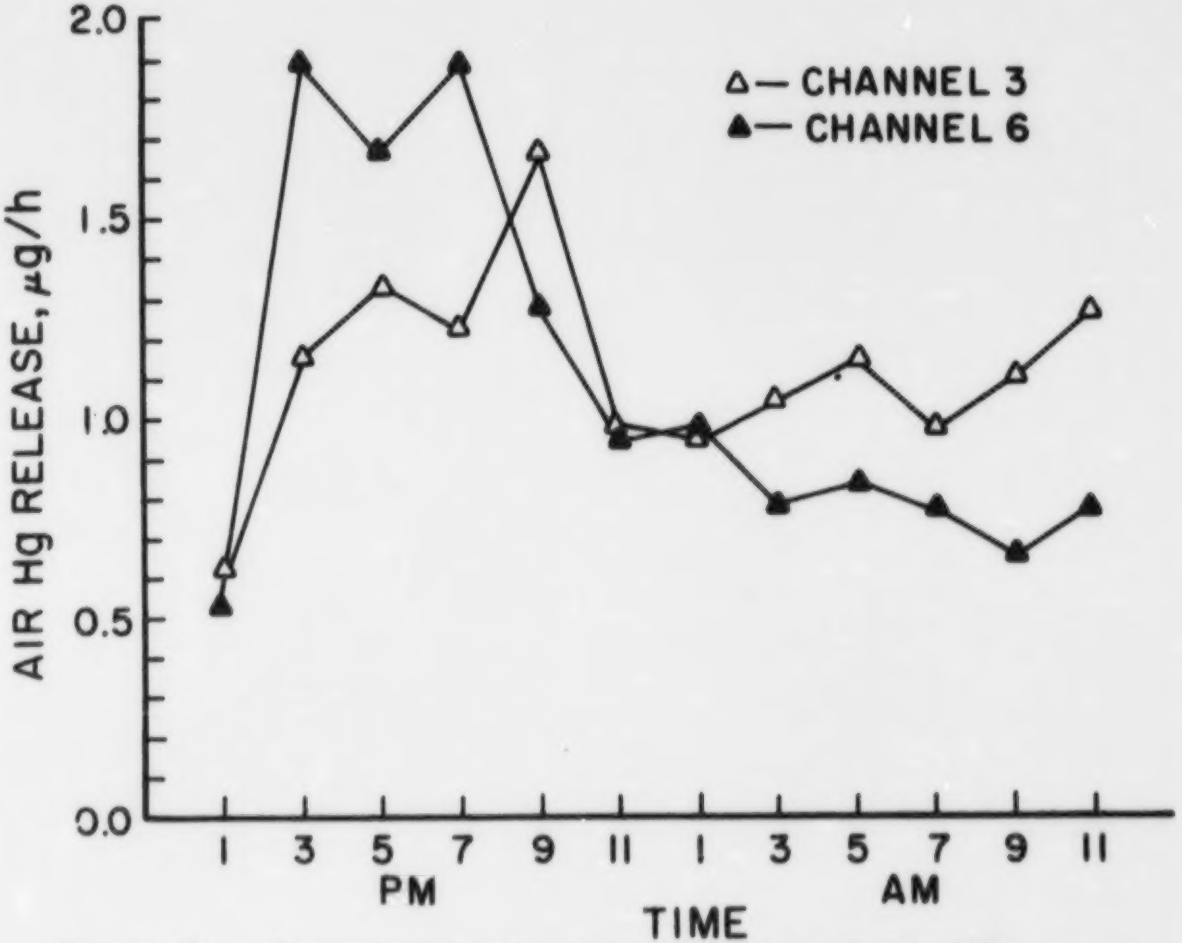

FIGURE 15. DIURNAL MERCURY RELEASES ON JANUARY 22-23, 1975, AT THE AIR-WATER INTERFACE OF CHANNELS RECEIVING AN INPUT OF 5.0 μg/l. THE AREAS SAMPLED WERE 0.186 M^2.

thickness of this mat was always greatest near the heads of the channels and became thinner toward the tails. Gradually this mat became less structured as emergent aquatic plants and larger animals, especially amphibian larvae, became established in the channels.

The sediment samples removed in the first part of the project consisted of frozen cores which were sectioned to give a vertical profile of Hg distribution. After several sets of samples, it became evident that all of the Hg in the cores was associated with the mat community covering the sand. Sand samples from the cores, and also from the benthic dishes used for invertebrate sampling, contained Hg levels of less than 0.2 µg/g except when they were contaminated with organic debris forced in by the coring procedure. At no time during the project were significant amounts of Hg found in the sand portion of the sediment. This was not a result of the drying process used because portions of mat were also dried and analyzed without a significant loss of Hg. Because of the localization of Hg in the organic layer on top of the sand, Hg values were calculated as µg/g ash-free dry weight of organic matter for comparisons between channels.

The results of analyses of the organic component of the channel sediments are presented in Figure 16 for the control and 0.01 µg/l treatments, and in Figure 17 for the 1.0 µg/l and 5.0 µg/l treatments. The data show that even in the channels receiving Hg inputs of 0.01 µg/l, there were elevated Hg levels in the sediments relative to the controls. When the 0.01 µg/l input was changed to 5.0 µg/l, there was a rapid increase in sediment levels although the data are insufficient to show whether or not an equilibrium level was reached. The sediment Hg levels in all treated channels showed an increase four months after the Hg inputs were stopped. There is no obvious explanation for this although it appears to be more than chance variability. There was no marked decrease in any other components of the channel communities at the time of the increase in the sediments.

Figures 16 and 17 clearly show the slow decrease in sediment levels in the 13-month period following the cessation of Hg input. The half-lives calculated from data collected after May, 1974 were 7.85 years ($C.I._{95\%}$ = 5.62-13.0 years) for the 1.0 µg/l treatments and 8.67 years ($C.I._{95\%}$ = 6.11-15.0 years) for the 5.0 µg/l treatments, assuming first order kinetics. These half-lives are considerably longer than the 1.3 years calculated by Bothner and

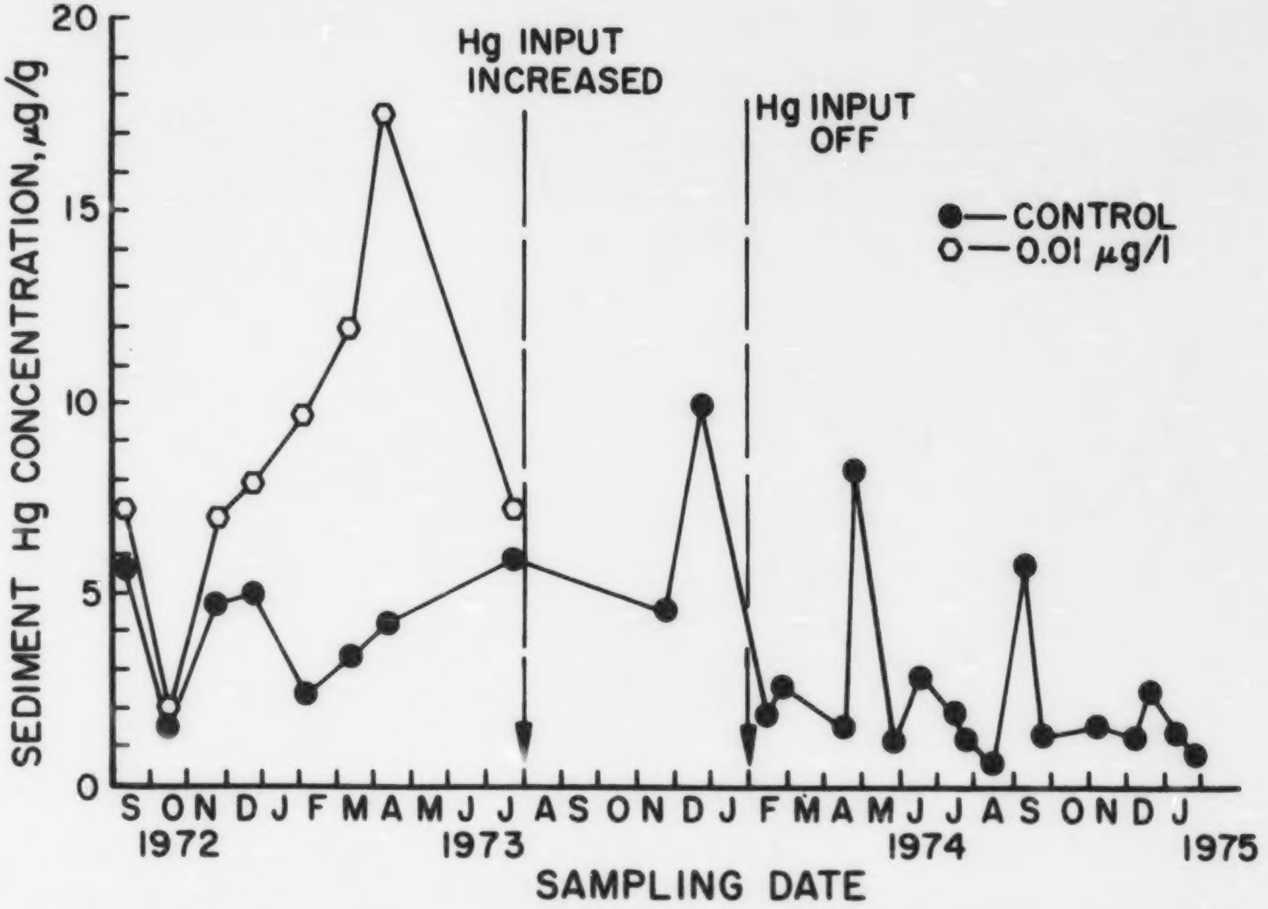

FIGURE 16. AVERAGE MERCURY CONCENTRATIONS IN THE ORGANIC PORTION OF THE SEDIMENTS OF THE CONTROL CHANNELS AND CHANNELS RECEIVING AN INPUT OF 0.01 μg/l.

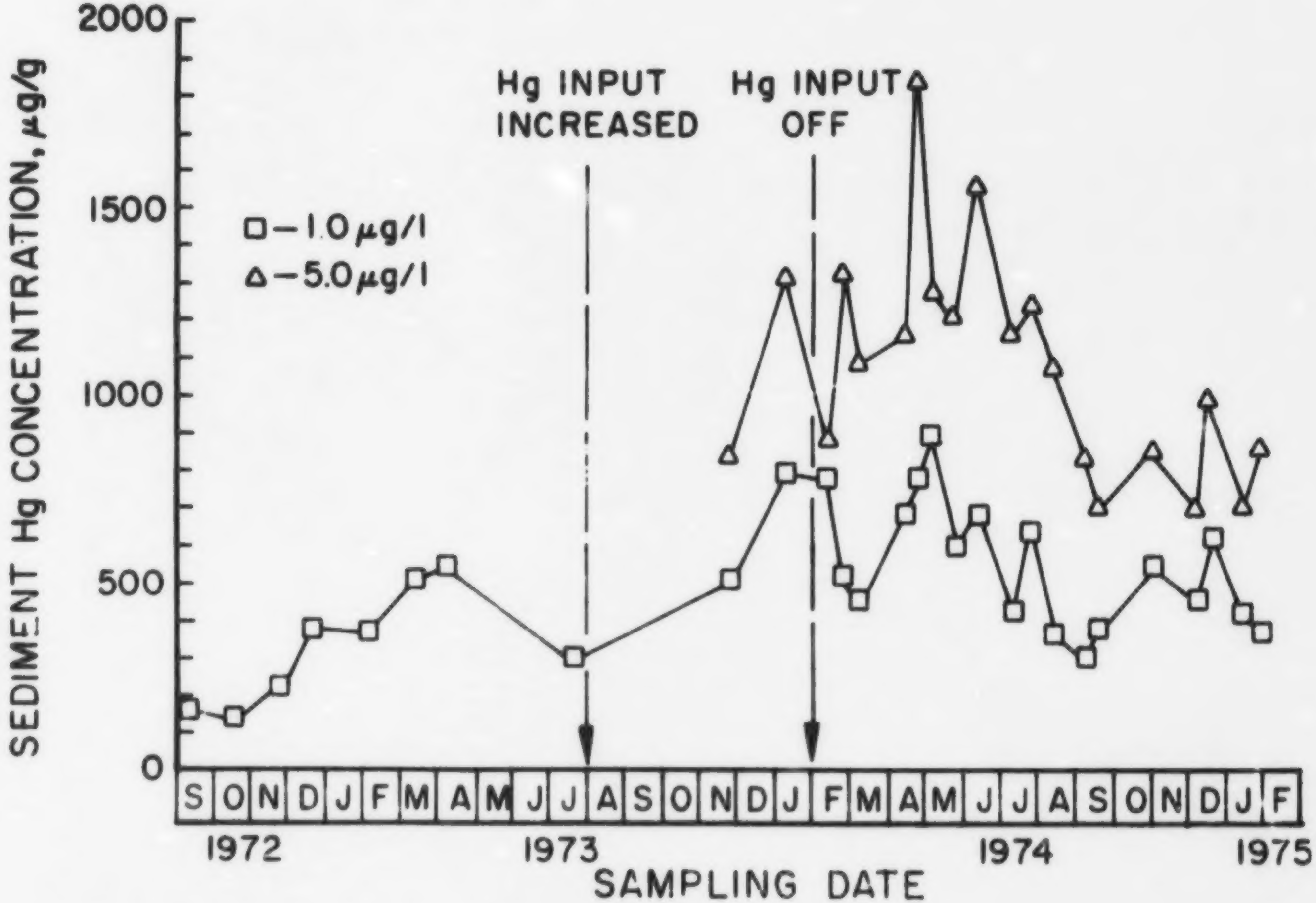

FIGURE 17. AVERAGE MERCURY CONCENTRATIONS IN THE ORGANIC PORTION OF THE SEDIMENTS OF CHANNELS RECEIVING INPUTS OF 1.0 AND 5.0 μg/l.

Carpenter (1974) for losses from a contaminated marine system but are similar to the values they calculated from the data of other workers (Spangler et al., 1973).

Export

Figure 18 summarizes the Hg concentrations measured in the particulate matter leaving the channels. Data for the control channels are not presented because of the generally low values (<4 µg/g ash-free dry weight) found in these samples. Although Figure 18 shows the typical high variability associated with the Hg analyses, several trends are evident. The Hg levels in export from channels 3 and 6 rose dramatically in the three-week period following the treatment changes in these channels from 0.01 to 5.0 µg/l. Indeed, it appears that an equilibrium level of approximately 1800 µg/g was reached in this short period of time. This concentration was a factor of only 2-3 times that measured in the 1.0 µg/l channels, even though the water levels were greater by a factor of 5.

Mercury concentrations were consistently higher in export material than in the sediments (Figure 17) until Hg inputs were stopped in January, 1974. Export levels then decreased more rapidly than the sediments and were much lower when the project was terminated. This indicates that the majority of the exported particulate was not derived from the sediments.

Periphyton

The most complete set of data for Hg levels in the channel periphyton is for material removed from the PVC lining. The results for the control channels and the channels receiving an input of 0.01 µg/l are presented in Figure 19. Figure 19 shows that Hg levels in the periphyton exposed to a water concentration of only 0.01 µg/l were higher than in samples from the control channels. It also shows that there was an increase in Hg concentrations in the control channels when the doses were changed in August of 1973, and a decrease in the control concentrations after the Hg inputs were stopped. Mercury was probably entering the control channels at the air-water interface since a series of wall periphyton samples taken every 20 m along the length of channel 1 (control) showed the Hg levels were about the same along the entire length. If there were a leak between adjacent channels, only areas downstream from the leak would have been affected.

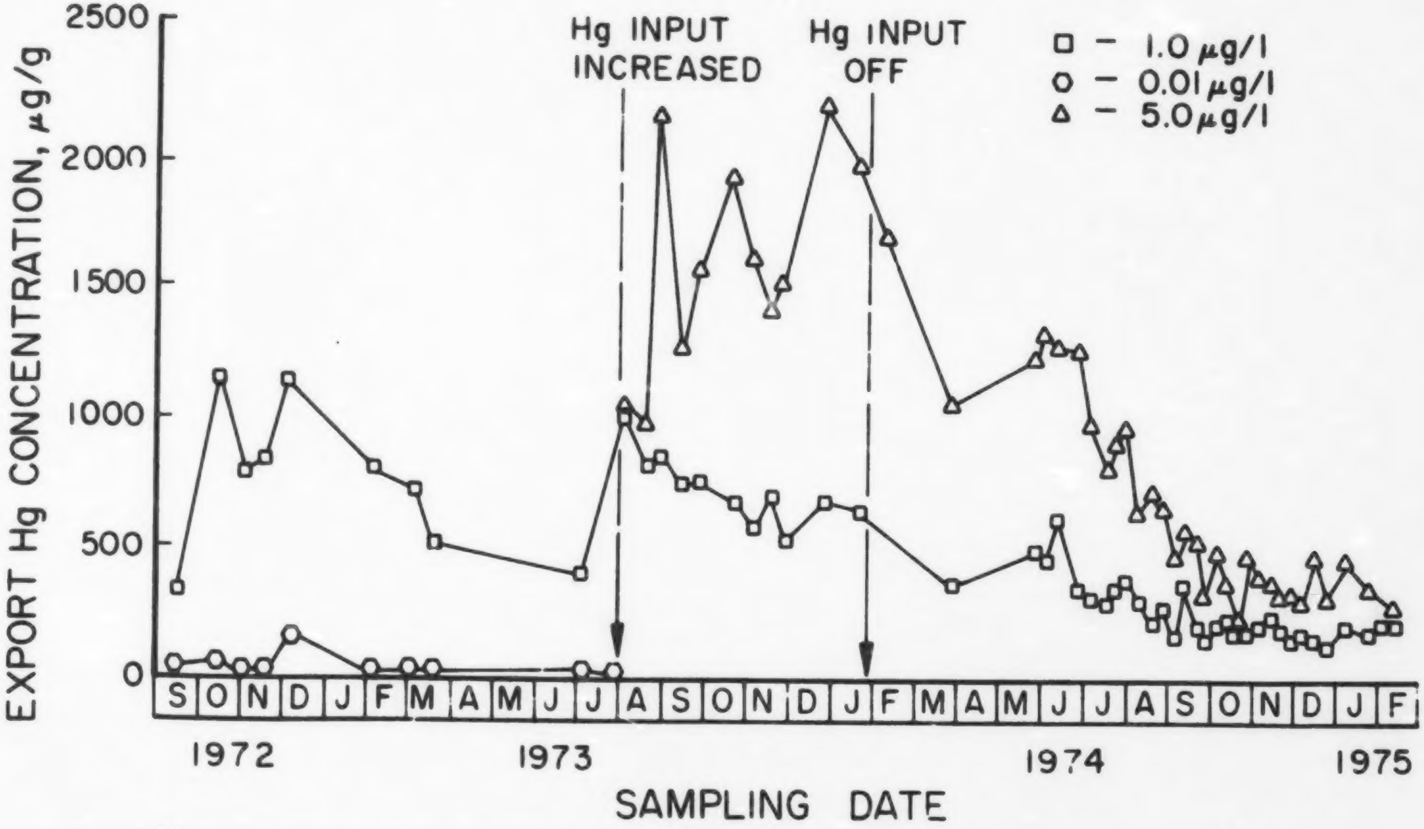

FIGURE 18. AVERAGE MERCURY CONCENTRATIONS IN PARTICULATE MATTER LEAVING THE TREATED CHANNELS.

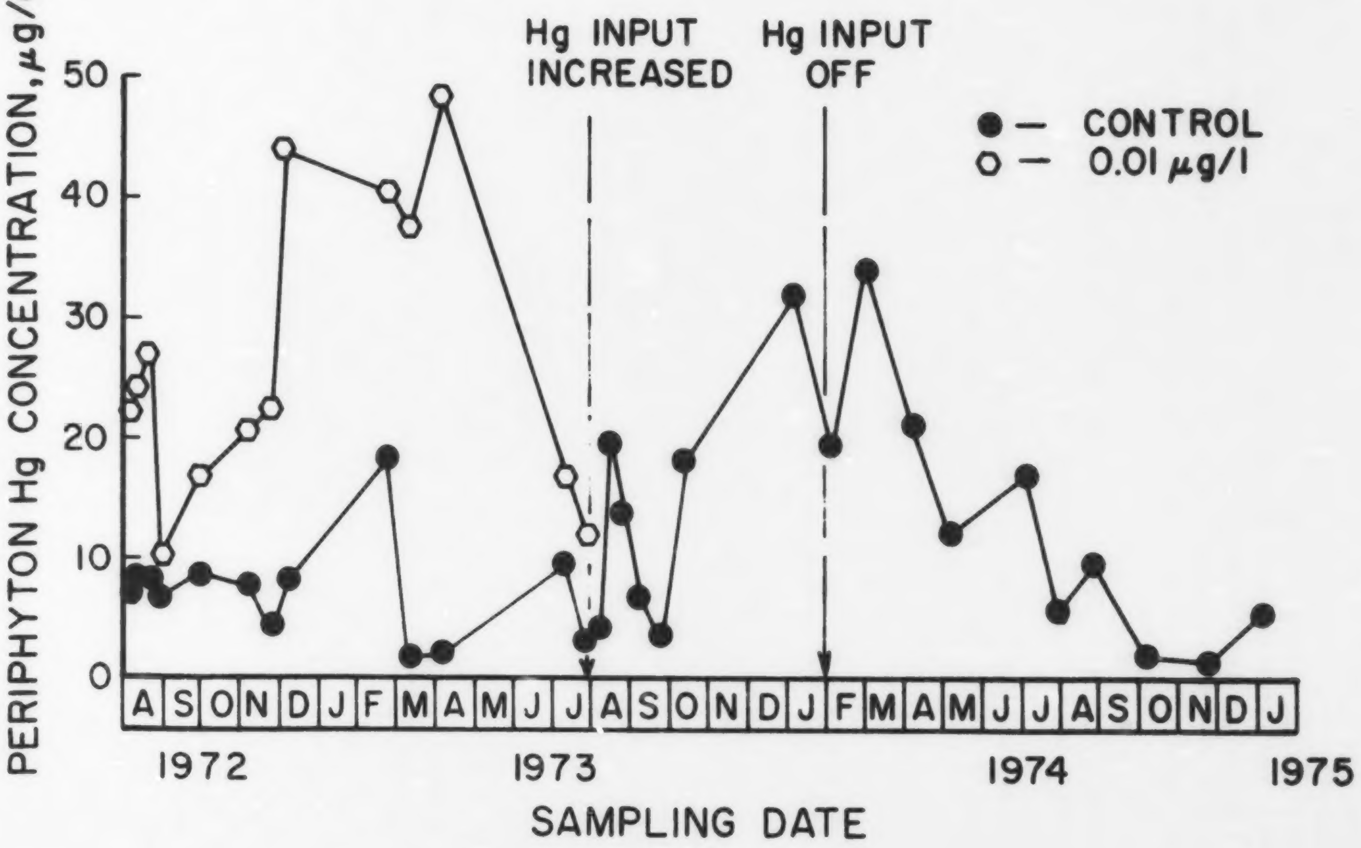

FIGURE 19. AVERAGE MERCURY CONCENTRATION IN PERIPHYTON REMOVED FROM THE PVC WALLS OF THE CONTROL CHANNELS AND CHANNELS RECEIVING AN INPUT OF 0.01 μg/l.

Results of the Hg analyses of periphyton removed from the PVC in the 1.0 and 5.0 μg/l channels are shown in Figure 20. The levels in the 1.0-μg/l channels reached a peak of 3000 μg/g after about seven months. They remained constant at this level for about five months and then, for no obvious reason, began to decrease until they reached 600 μg/g by the time the Hg was turned off. During the last six months of the project, the levels remained relatively constant at 150-200 μg/g. This was slightly lower than the concentration measured in the export leaving the channels during this same period (Figure 18) which indicates at least some contribution to the export from the sediments.

Figure 20 shows that the periphyton Hg levels in the 5.0 μg/l channels were very erratic. The high value observed in October, 1973, which like all other points is an average of four samples, appears to be a valid measurement since all four samples were high. After this peak, the decrease in levels in the 5.0-μg/l channels parallels the observations from the 1.0-μg/l channels, although the values reached just prior to the end of the project were slightly higher.

Data for periphyton removed from the plexiglass plates are summarized in Figure 21. Periphyton removed from the plexiglass plates always had much higher Hg levels than that scraped from the PVC walls. This difference was maintained until the end of the project when periphyton removed from the plexiglass plates had concentrations of approximately 1000 μg/g and PVC periphyton had concentrations of 200 μg/g. The highest Hg levels measured in plexiglass plate samples (>8000 μg/g in January, 1974) were still lower than those reported by Matida and Kumada (1969) for attached algae samples taken from a contaminated river in Japan. Concentration factors calculated from the maximum periphyton levels measured in plexiglass plate samples range from 1×10^4 to 3×10^4. This range is considerably higher than the values reported by Hannerz (1968) for algae from experimental ponds but is very similar to the range of concentration factors reported by Fujita and Hashizume (1972) for a freshwater diatom.

Although the water levels of Hg during the last year of the project were below detection limits, periphyton colonizing new plexiglass plates put into a channel formerly treated with Hg levels of 5 μg/l in June, 1974, acquired equilibrium Hg concentrations of 45 μg/g after two months.

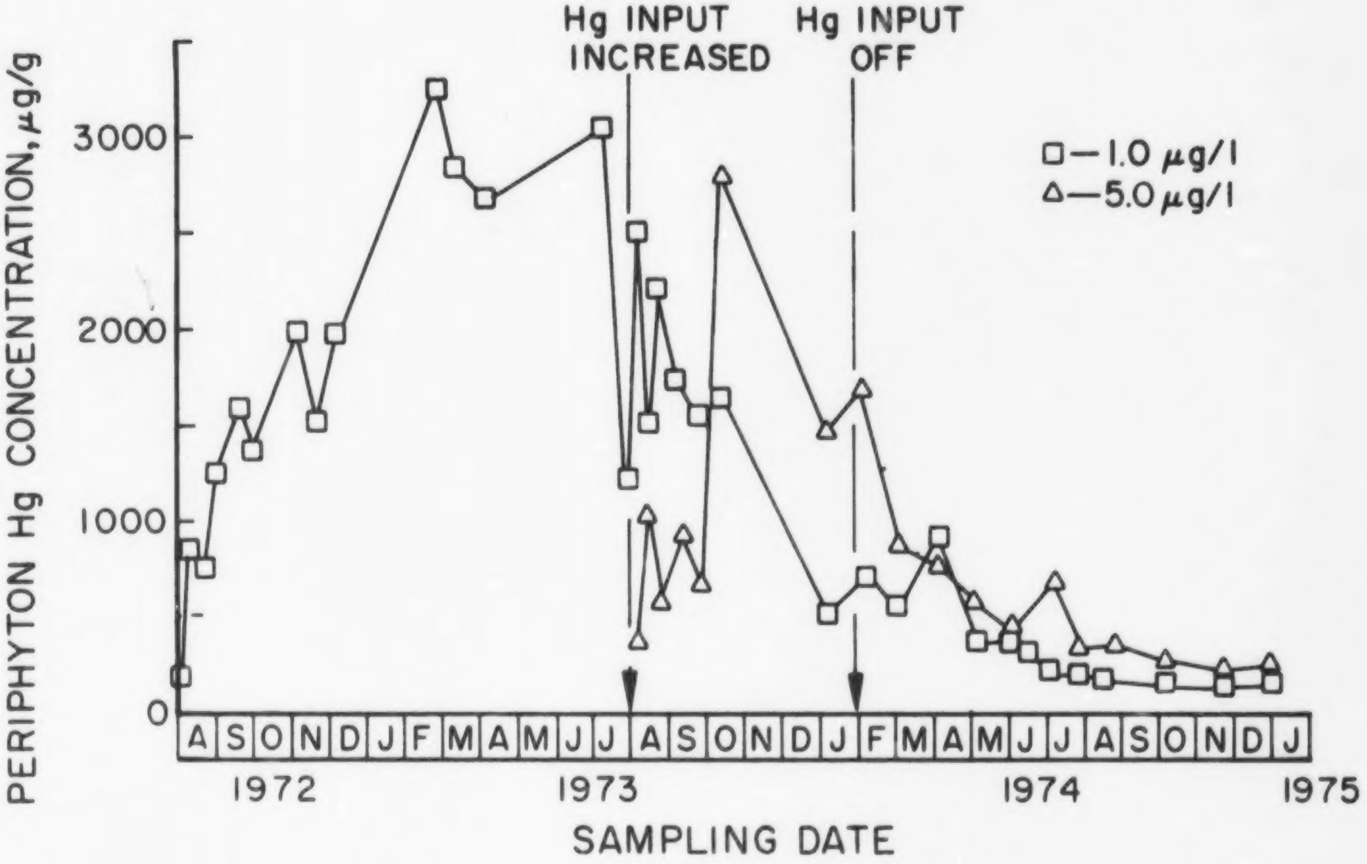

FIGURE 20. AVERAGE MERCURY CONCENTRATIONS IN PERIPHYTON REMOVED FROM THE PVC WALLS OF CHANNELS RECEIVING INPUTS OF 1.0 AND 5.0 $\mu g/l$.

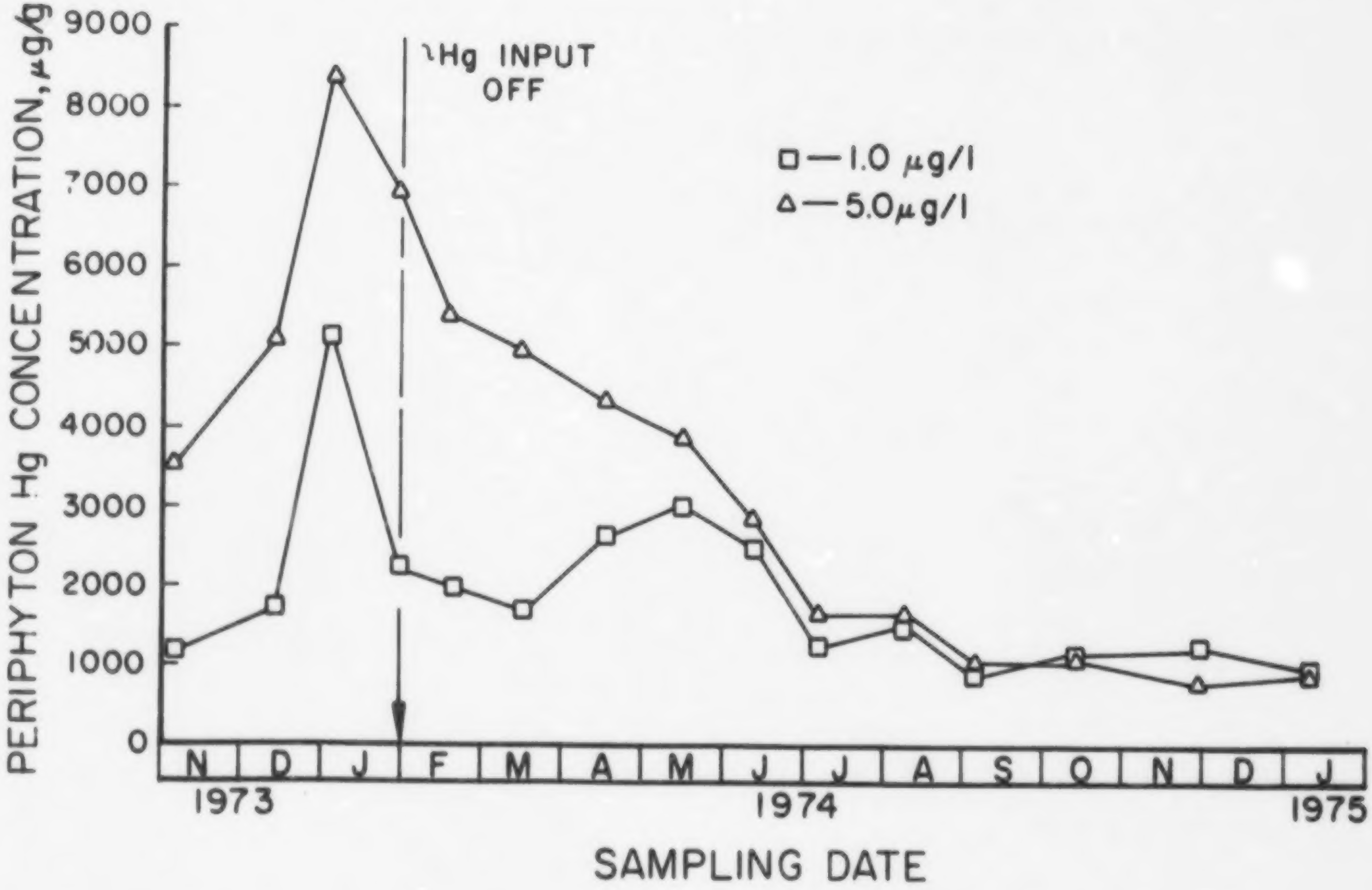

FIGURE 21. AVERAGE MERCURY CONCENTRATIONS IN PERIPHYTON REMOVED FROM PLEXIGLASS PLATES IN CHANNELS RECEIVING INPUTS OF 1.0 AND 5.0 $\mu g/l$.

Macrophytes

In the first 18 months of channel operation, the bottom communities were relatively simple with no rooted vegetation. By the spring of 1973, however, a great number of small rooted plants were noted, especially in the upstream portions of the outer channels. Although there appeared to be two distinct species, these were later identified as variants of a single species, *Juncus diffusissimus*. The distribution of this plant in the channels in April of 1973 is shown in Table 3. Quite obviously, channel one received a much greater input of seeds than the other channels. The upstream-downstream gradient in the distribution pattern may be due to the input of seeds to the pools above the channels.

Starting in June, 1973, samples of *Juncus* were periodically analyzed for Hg content. Results of root analyses are shown in Figure 22, results of leaf analyses are shown in Figure 23. No data are presented for the control channels or the channels receiving an input of 0.01 μg/l because the values were so low. In all cases, when Hg was being input to the channels, the levels in the leaves were higher than in the roots. The root levels were significant, however, indicating movement of Hg within the plants since the sand in which the roots were growing had an extremely low Hg content. The rapid increase in root levels during the spring of 1974, several months after the Hg inputs were stopped, also indicates internal shifting of Hg. The Hg in the leaves of the *Juncus* appears to have been tightly bound since there was little Hg loss from the leaves for several months after the inputs were stopped. The rapid decrease in leaf concentrations beginning in May was probably due to dilution by growth and the inclusion of newly grown plants, which had not been exposed to Hg, in the samples.

During the latter part of the period when Hg was being input, the levels in the *Juncus* leaves were in approximately the same ratio (1:5) as the water levels. This is one of the few components of the channel communities that showed this relationship. At the termination of the project, Hg levels in the roots and stems were approximately equal in both treatments, and were declining very slowly.

Concentration factors calculated from the maximum Hg levels reached in the stems during the project are on the order of 2.5×10^3. The conversion factor for estimating wet-weight concentrations from the ash-free dry weight data was 0.13 and was derived experimentally from *Juncus* removed from the

Table 3. DISTRIBUTION OF JUNCUS DIFFUSISSIMUS ON APRIL 2, 1973, IN THE ARTIFICIAL STREAMS
(number of plants/interval)

	Channel number					
Distance from input weir, meters	1 control	2 1.0 µg/l	3 0.01 µg/l	4 control	5 1.0 µg/l	6 0.01 µg/l
0-7.5	453	112	30	170	139	294
7.5-15	261	131	8	39	10	40
15-22.5	144	30	8	16	9	20
22.5-30	98	23	3	10	5	11
30-37.5	97	13	3	11	8	5
37.5-45	124	18	11	34	18	13
45-52.5	222	31	20	33	25	16
52.5-60	70	13	3	6	1	3
60-67.5	38	6	0	2	4	2
67.5-75	26	0	3	7	1	5
75-82.5	49	17	2	2	12	5
82.5-90	236	18	5	31	5	9
Total	1818	412	96	361	237	423

channels. The concentration factors determined by Hannerz (1968) for rooted aquatics seldom exceeded 100, although Dietz (1972) calculated values > 430 for submerged spermatophytes.

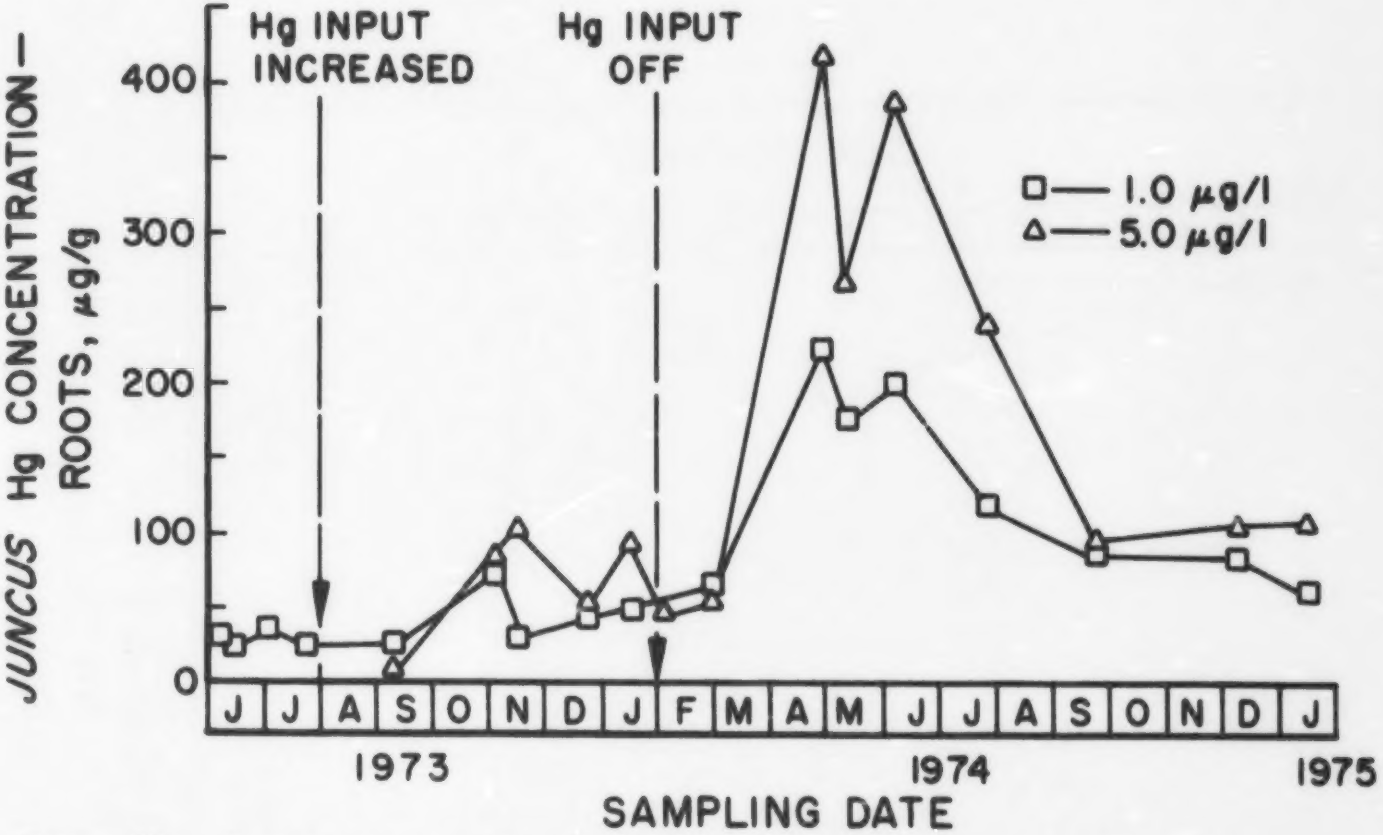

FIGURE 22. AVERAGE MERCURY CONCENTRATIONS IN ROOTS OF ___JUNCUS DIFFUSISSIMUS___ REMOVED FROM CHANNELS RECEIVING INPUTS OF 1.0 AND 5.0 $\mu g/l$.

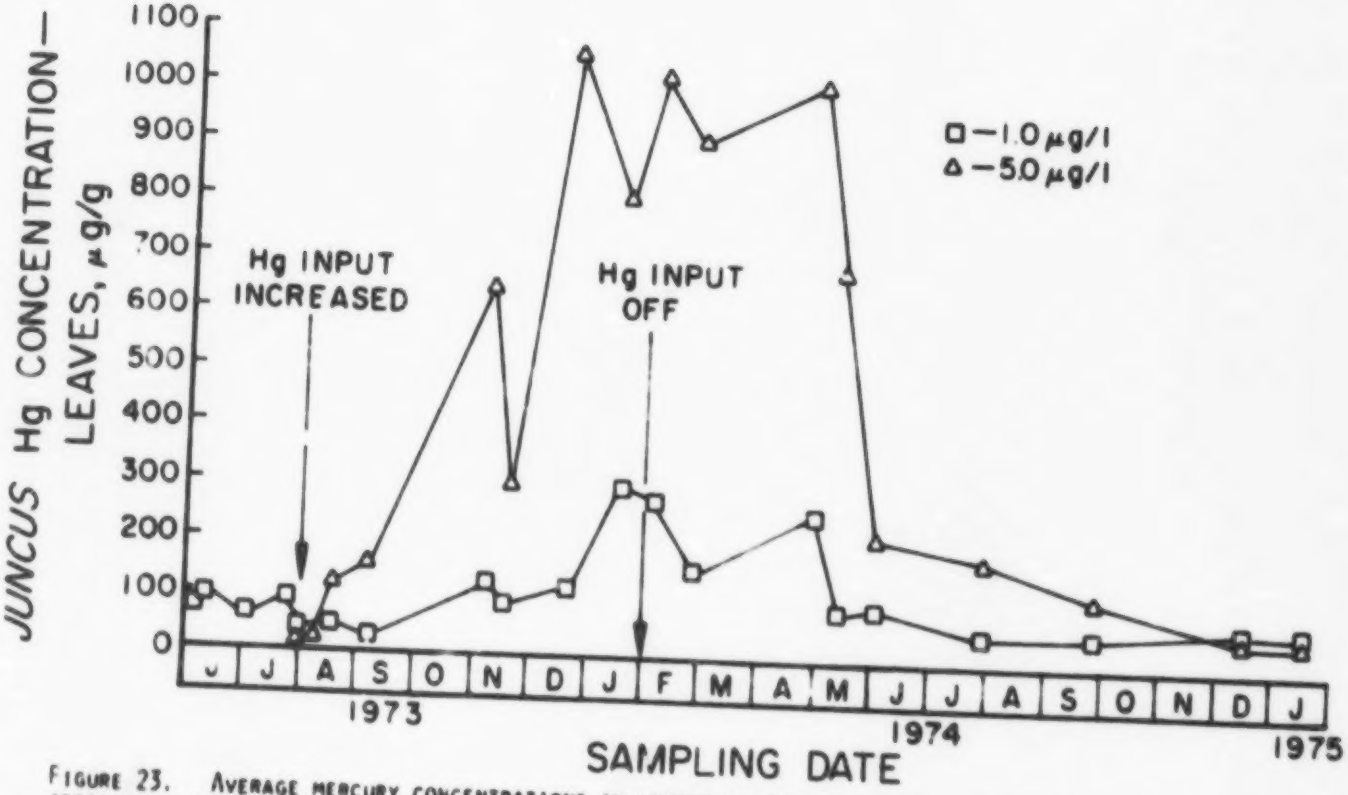

FIGURE 23. AVERAGE MERCURY CONCENTRATIONS IN LEAVES OF JUNCUS DIFFUSISSIMUS REMOVED FROM CHANNELS RECEIVING INPUTS OF 1.0 AND 5.0 µg/l.

Aquatic Insects

Early in the study, the larger aquatic insects were relatively rare in the channels and no attempt was made to collect these on a routine basis. During the entire project period, the organisms removed from the benthic dishes were never available in sufficient numbers to provide an adequate biomass for Hg analyses. Also, the taxonomic problems associated with the groups most common in the benthic samples made it impossible to identify and enumerate these organisms without first preserving them.

The number of species and population densities of aquatic insects increased as the rooted macrophytes became an important channel component. During the last 14 months of the project, the material collected in the fish-retaining end screens was periodically searched for macroinvertebrates for analysis. The organism for which most data were collected was a damselfly of the genus Ischnura. The data are summarized in Figure 24 in which each point represents the average of two to nine analyses, each of an individual nymph. The average coefficient of variation (CV) associated with the results from the 1.0-µg/l channels was 46% and from the 5.0-µg/l channels, 40%. Results for Ischnura collected from the control channels are not shown because the numbers were typically very low (<0.1 µg/g) and also variable (CV=116%). Figure 24 shows that the Hg levels in this damselfly were probably a result of direct water uptake. The concentrations in animals collected in the two treatments were approximately in the same ratio as the water levels (5:1) and these concentrations converged rapidly to low levels after the Hg inputs were stopped.

During the Hg input period, another damselfly, Argia sp., was collected occasionally from all channels other than those receiving an input of 5.0 µg/l. Based on a sample of six animals collected from the 1.0-µg/l channels during January, 1974, the mean Hg level of 12.8 µg/g (SD=5.7) for this damselfly was about the same as that found in Ischnura sp. taken from the same channels at about the same time. Argia collected from the same channels in February and March of 1974 had levels that ranged from 3-5 µg/g, values very similar to those observed for Ischnura. In the time interval between the Hg shut-off and the project termination, Argia became established in channels 3 and 6 and at the end of the project, levels measured in this species were 0.25, 1.7 and 2.2 µg/g respectively for animals from the control, former 1.0-µg/l and former-5.0 µg/l channels. Levels in Ischnura at the end of the study

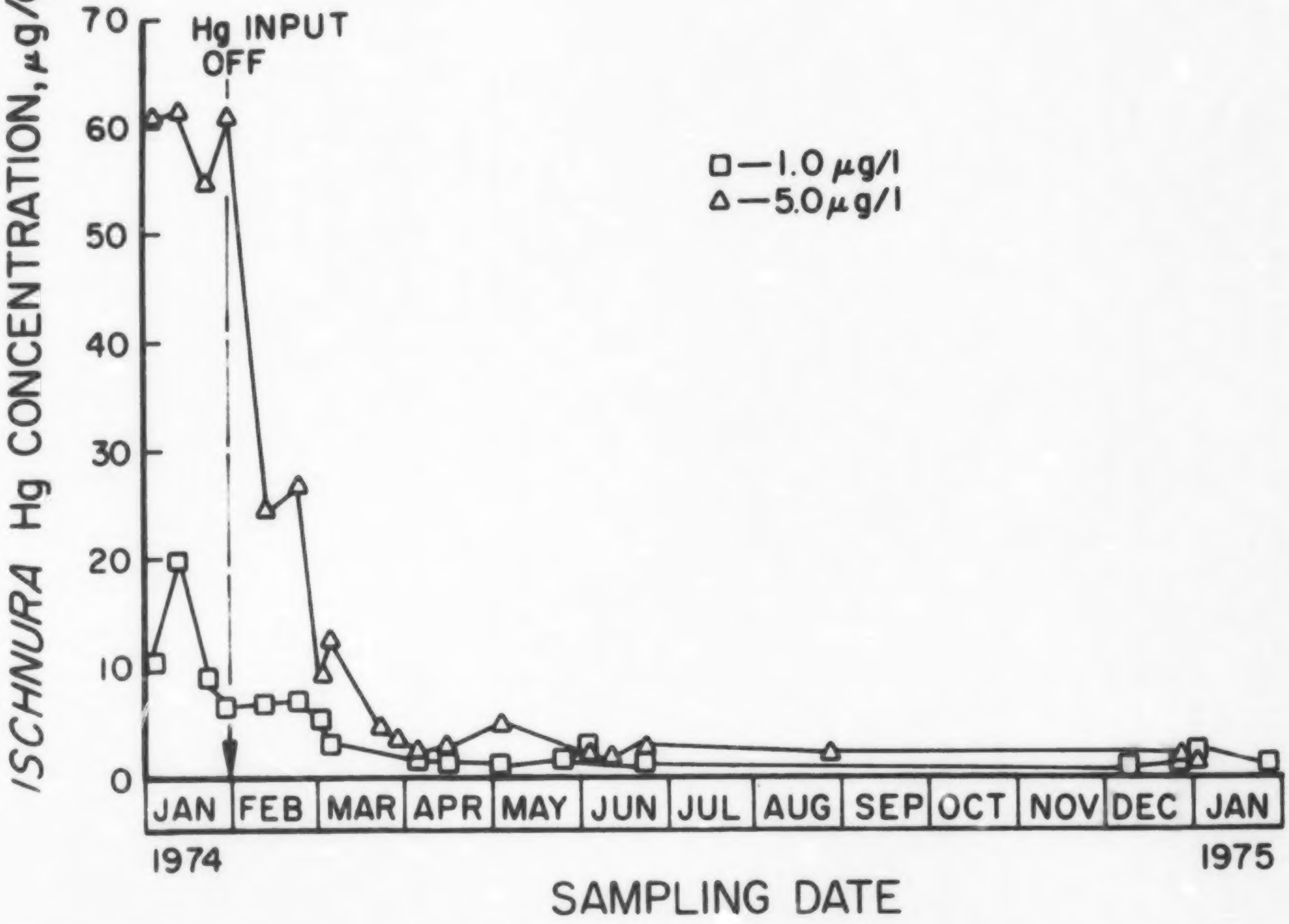

FIGURE 24. AVERAGE MERCURY CONCENTRATIONS IN DAMSELFLY NYMPHS (*ISCHNURA* SP.) COLLECTED FROM CHANNELS RECEIVING INPUTS OF 1.0 AND 5.0 μg/l.

were 0.09, 2.6 and 2.8 μg/g in these same channels, again indicating the two species were quite similar with respect to Hg uptake and elimination.

Analyses were made of other immature insects, including several other species of odonates, dipterans, aquatic lepidopterans and several species of adult coleopterans, notonectids and corixids. Levels for other odonate nymph species were about the same as those presented for Ischnura and Argia. Values for the adult forms (notonectids, coleopterans and corixids) were extremely variable, probably as a result of movement of these groups in and out of the channels. During the course of the project, corixids and other adult insects were seen flying from one channel to another. In January, 1974, corixids were observed to be very common in the channels and 18 individuals (Hesperocorixa sp.) were collected, three from each channel. Mean values ($\bar{X}$ ± SD) for the Hg concentrations in the three treatments were 0.11 ± 0.13 μg/g, 0.34 ± 0.20 μg/g and 3.91 ± 5.5 μg/g for the controls, 1.0 μg/l and 5.0 μg/l treatments, respectively.

In August, 1974, a large number of uncontaminated Hesperocorixa were collected from a nearby pond. These animals were placed in the continuous-flow chambers previously described to determine their ability to take up Hg directly from the water and to measure the variability of their concentrating ability under controlled conditions. The results are presented in Figure 25. The relatively rapid uptake by the unfed animals indicates that direct uptake by this species was of major importance in the channels. The lower variability in the CFC-treated animals in contrast to the channel organisms was probably due to the fact that they were confined to a given treatment and there was no exchange between treatments as took place in the channels.

Midge larvae were an important link in the food web of the channel communities, and were numerically the most abundant invertebrates. Because of the small size of the individual organisms, composite samples of a number of individuals were made for Hg analyses. Average concentrations in midge larvae at the time the Hg inputs were terminated ranged from below detection limits in the control channels to 8 μg/g in the 1.0-μg/l channels and 20 μg/g in the 5.0-μg/l channels.

The concentrations of Hg found in the channel insects were much lower than those found by Matida and Kumada (1969) in

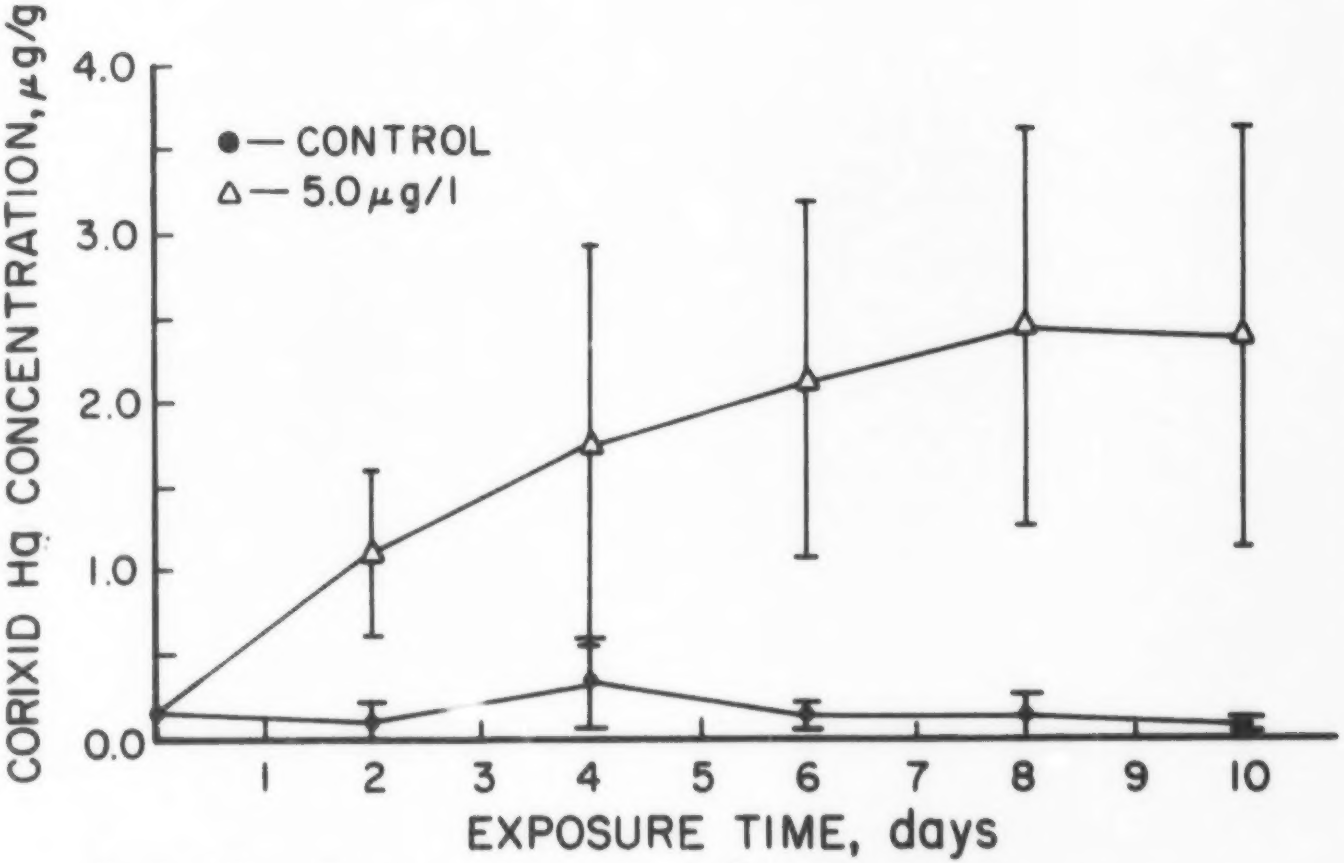

FIGURE 25. MERCURY CONCENTRATIONS ($\bar{X}$ ± 1SD) ACQUIRED BY CORIXIDS (HESPEROCORIXA SP.) CONFINED TO FLOWING WATER CONTAINING 5.0 µg/l. Hg^{++}. EACH POINT IS BASED ON ANALYSES OF 10 ORGANISMS.

a contaminated Japanese river although the concentration factors that can be calculated from the channel results, are much higher than those reported by Hannerz (1968) for his pond experiments with $HgCl_2$.

Fish

The background level of Hg in the mosquitofish collected for this project, as determined in April, 1972, was 0.04 ± 0.01 µg/g ($\overline{X} \pm 2SE$, n=20). This value is much lower than the 0.22 µg/g (wet weight) level in the experimental mosquitofish used by Schoper (1974). The pond from which the fish for this project were taken is located adjacent to an installation on the SRP which consumes a large quantity of coal. The large stack releases from this installation do not appear to have affected the pond system, although other components have not been checked.

During the first year of the project, a number of fish escaped from both the channels and cages when the screens at the ends of the channels plugged and water levels became excessive. The reduced numbers of fish made sampling difficult and also greatly limited the number of samples that could be taken. It was for this reason that all remaining fish were removed at the end of the first year and new ones introduced.

The results of analyses of fish removed from the 1.0-µg/l channels (2 and 5) between May, 1972, and May, 1973, are presented in Figure 26. Fish in channel 2 acquired Hg faster and to a greater extent than those in channel 5. In May, 1973, the average Hg concentration in the 21 fish removed from channel 2 was 5.30 ± 0.96 µg/g ($\overline{X} \pm 2SE$), in the 62 fish removed from channel 5, 3.41 ± 0.30 ($\overline{X} \pm 2SE$). The means are significantly different ($p \leq 0.01$) based on a t-test of the log transformed (to uncouple the variance and mean) data. There is no apparent reason for the differences between channels. The fish population in channel 5 was greater than in channel 2 but the average weight of the fish did not differ (0.52 g in channel 2, 0.57 g in channel 5) when the populations were removed in May, 1973.

Figure 26 shows that the Hg levels in the mosquitofish exposed to 1.0 µg/l rose slowly over a 10-month period to equilibrium levels of 4-7 µg/g. There may be a seasonal cycle indicated, with a peak occurring during the winter months, especially if the values for the fish collected during the population removal in May, 1973, are included.

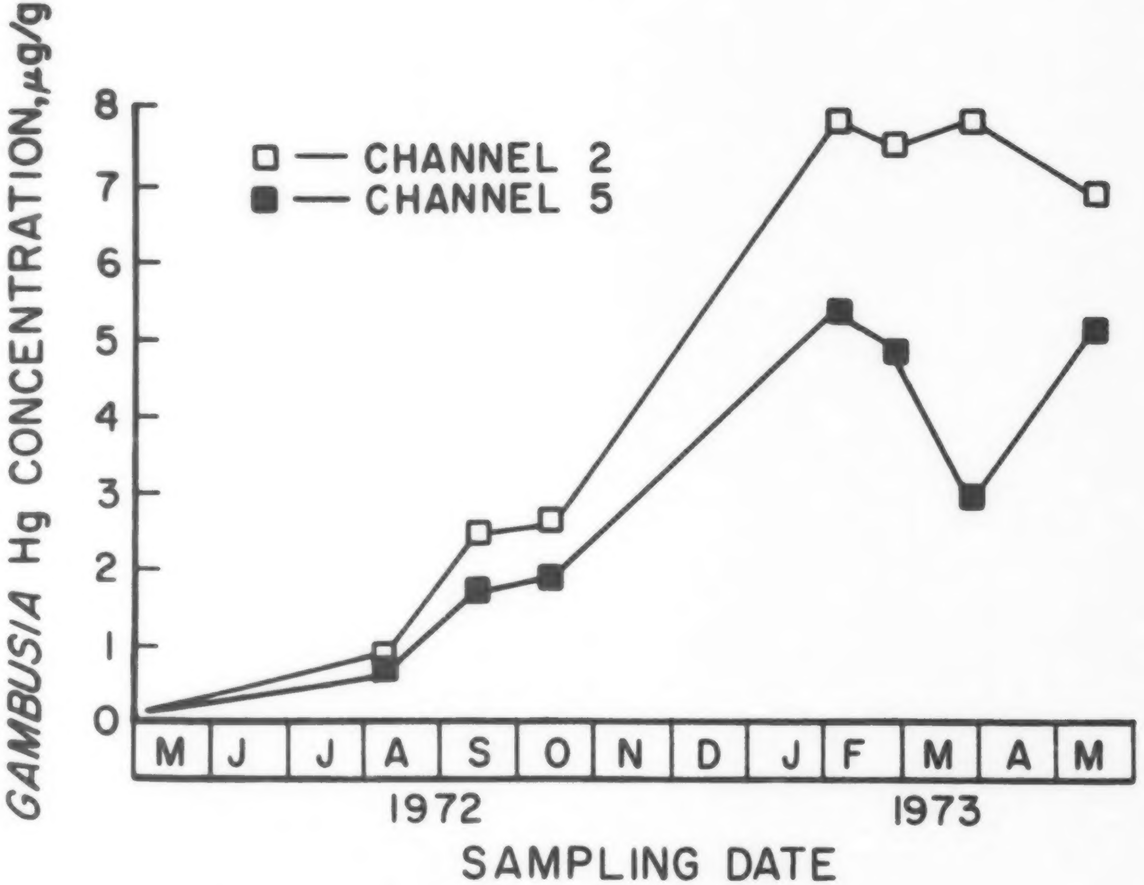

FIGURE 26. MERCURY CONCENTRATIONS IN MOSQUITOFISH (*GAMBUSIA AFFINIS*) REMOVED DURING THE FIRST YEAR FROM THE CHANNELS RECEIVING AN INPUT OF 1.0 μg/l. EACH POINT IS AN AVERAGE BASED ON TWO ANALYSES OF INDIVIDUAL FISH.

A similar seasonal trend is indicated in Figure 27 which summarizes the results of analyses of fish removed from the control channels and those receiving an input of 0.01 µg/l Hg. Figure 27 shows that at levels of even 0.01 µg/l, mosquitofish accumulate Hg above control levels. The increase in the control fish is probably due to air transfers of Hg between channels although this may be a natural seasonal phenomenon that has simply not been observed before. Statistical tests of measurements made on the fish removed from the control and 0.01-µg/l channels show that there were no significant differences in Hg levels and weights of fish between channels treated alike, but that there was a significant ($p < 0.01$) difference between the Hg concentrations in the control fish ($0.072 \pm .009$, $\bar{X} \pm 2SE$, n=34) and those removed from the 0.01-µg/l channels (0.179 ± 0.007, $\bar{X} \pm 2SE$, n=99).

The Hg levels in fish removed from the control channels after 1 year were significantly ($p < 0.01$) higher than those measured in the fish at the beginning of the project. This may reflect a natural increase with age, or it may be due to cross contamination between channels, as mentioned above. The differences in population sizes between channels can in no way be attributed to treatment since so many fish escaped from the channels.

Linear correlation coefficients between the body weights and the Hg concentrations in fish were calculated on a channel basis for all fish sampled between May, 1972, and May, 1973. Only in channel 6 was there a significant ($r=0.82$, n=12, $p < 0.01$) correlation. Correlation coefficients for the same variables were calculated with data collected from the fish populations removed from the channels in May, 1973. There was a significant negative correlation ($r=-.61$, n=21, $p < 0.01$) for animals removed from channel 2. There does not appear to be a neat, consistant relationship between weight and Hg levels in mosquitofish exposed to low levels of Hg over a long time period. This is not consistent with the data reported by Johnels et al. (1967), Johnels and Westermark (1970), Fimreite et al. (1971), Knight and Herring (1972) and Kelso and Frank (1974) although Scott and Armstrong (1972) found all possible relationships (positive, negative, and zero correlation coefficients) between Hg levels and fish length, depending on what species they were considering.

At the end of one year of exposure, there was no difference between the Hg levels in fish living in the channels and those confined to cages and fed with a commercial tropical

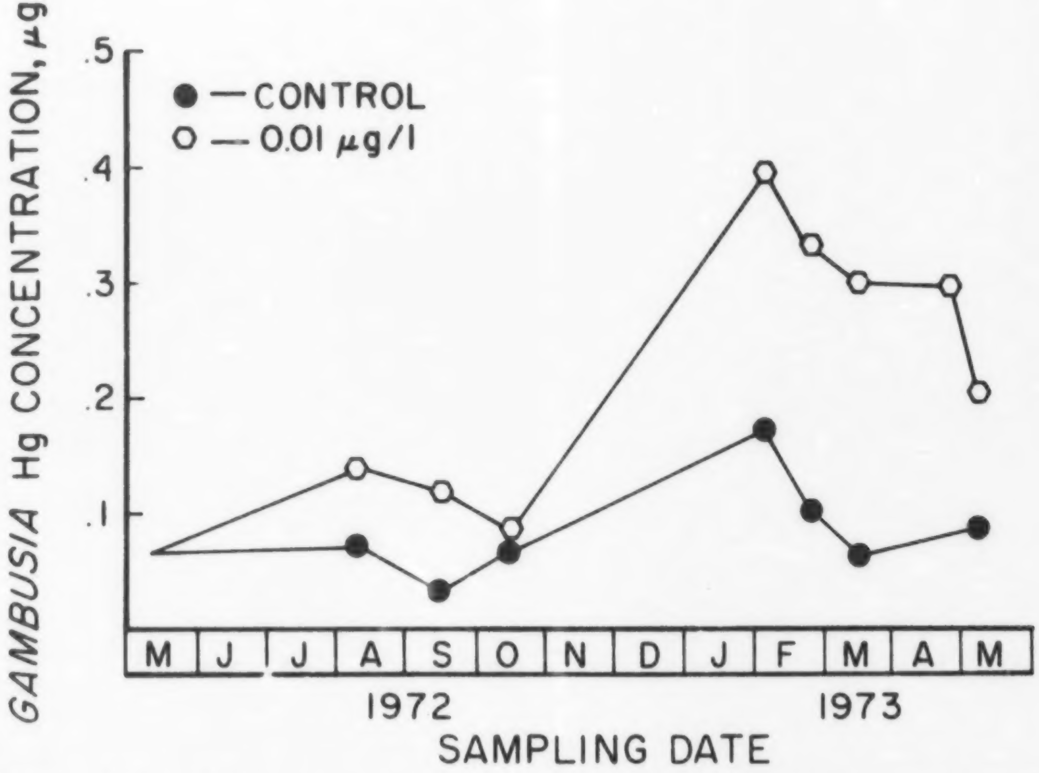

FIGURE 27. MERCURY CONCENTRATIONS IN MOSQUITOFISH (GAMBUSIA AFFINIS) REMOVED DURING THE FIRST YEAR FROM THE CONTROL CHANNELS AND THE CHANNELS RECEIVING AN INPUT OF 0.01 μg/l. EACH POINT IS AN AVERAGE BASED ON FOUR ANALYSES OF INDIVIDUAL FISH.

fish food containing 0.20 ± 0.02 μg/g Hg on a dry weight basis. This indicates that direct uptake of Hg from the water makes a significant contribution to the body burdens of mosquitofish. Based on two sets of analyses on composite samples of several fish, the portion of the total Hg present as methyl mercury was 28% in mosquitofish exposed for one year. This value is low compared to published results (Kamps et al., 1972; Bache et al., 1971) probably because the entire fish was digested for total Hg (including gut contents) rather than only the muscle tissue of the fish.

Fish data collected the last 20 months of the project from fish placed in the channels in June, 1973, are summarized in Figure 28. Mercury levels in fish from the control channels ranged from 0.005 to 0.17 μg/g. The data are averaged for treatments since there were no significant differences between channels treated alike during this period.

Figure 28 shows that the fish in the 1.0-μg/l channels acquired about the same Hg concentrations as the fish of the previous year. This equilibrium level was reached more quickly however, (6 months as opposed to 10), which indicates that fish placed in a contaminated area concentrated Hg faster than those placed in a clean area that was then contaminated. This result implies that direct uptake of Hg^{++} from the water is not the only method by which mosquitofish acquire Hg. Food chain uptake may be involved, although the conversion of Hg^{++} to elemental Hg, a process already mentioned as occurring in the channels, may also be important. Schoper (1974) found that elemental Hg is more rapidly taken up by mosquitofish than Hg^{++}.

Figure 28 shows the rapid increase in Hg levels in fish living in the channels when the concentrations were changed from 0.01 μg/l to 5.0 μg/l. The equilibrium level of 11-14 μg/g in the fish was reached in only four months. Although the Hg concentrations in the water of the treated channels were in the ratio of 5:1, the levels reached by the fish were only 2:1. Hence the concentrations attained by fish were not a simple linear function of water concentrations.

The levels in all treated fish decreased to approximately 3 μg/g within three months after the Hg inputs were turned off. These data are variable but indicate that biological half-lives may be a function of the initial concentration levels of Hg. From May, 1974, until the end of the project,

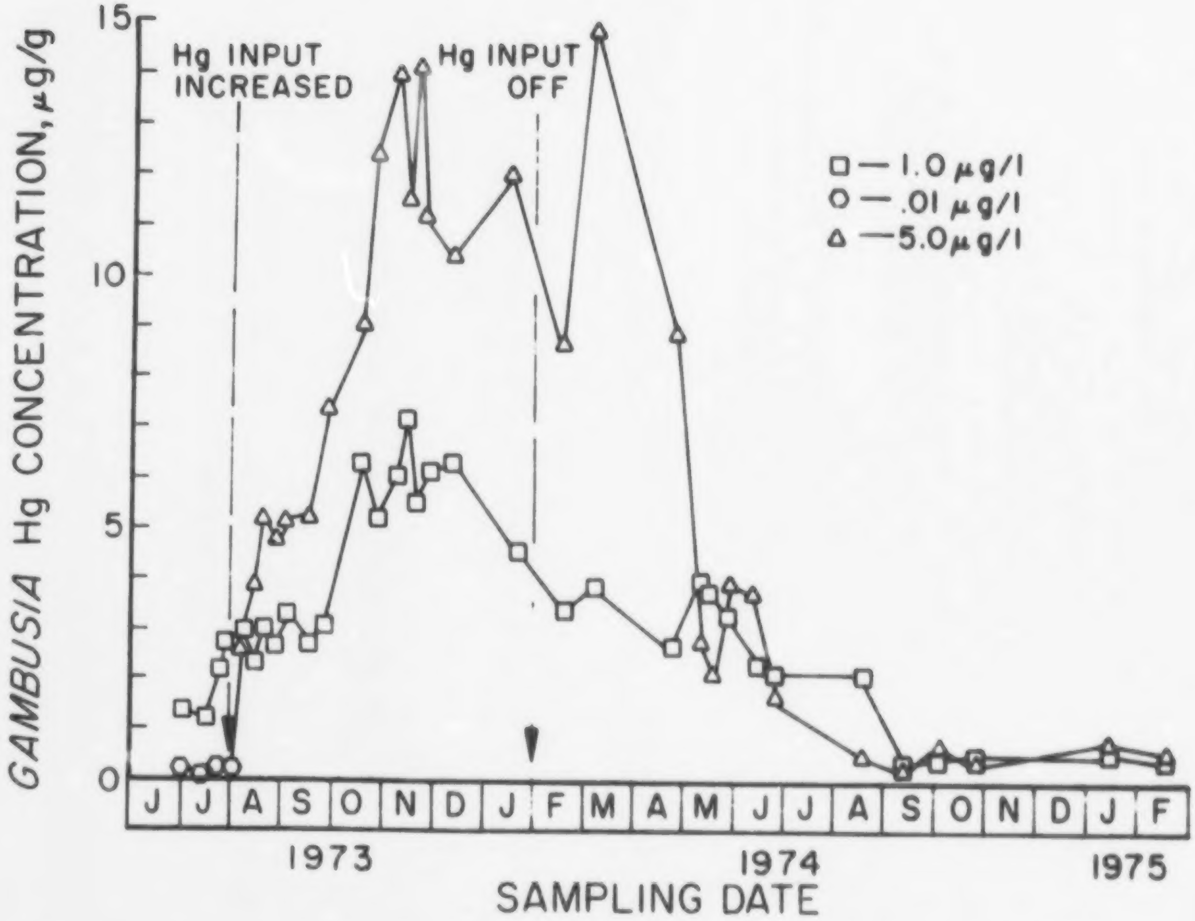

Figure 28. Mercury concentrations in mosquitofish (*Gambusia affinis*) removed from the treated channels during the last 21 months of the project. Each point is an average based on four analyses of individual fish.

there were no differences between fish exposed to the two treatment levels. By September, 1974, an equilibrium was reached in the contaminated fish at a level significantly above that measured in the controls. This level was maintained until the project was terminated in February, 1975. At that time the average concentration in fish from the treated channels was approximately 0.4 μg/g. Only a small fraction of the fish recovered at the end of the project have been analyzed.

During the project period included in Figure 28, there was successful mosquitofish reproduction in the channels. The increased community complexity that developed provided the necessary cover for young animals. Fish collected during this interval (June, 1973-March, 1975), might have been born in the channels. This was not true for fish collected the first year.

A single set of methyl mercury analyses was made on composite samples of fish removed from the channels in August, 1973. Although the total Hg concentrations ranged as high as 5.6 μg/g, the portion of Hg present as methyl mercury was on the order of 10%, considerably lower than the 28% value calculated for fish removed from the channel after a full year. This suggests that the proportion of total Hg present in fish as methyl mercury increases with exposure time. This result was also observed by Bache et al. (1971) who found that the proportion of methyl mercury to total Hg increased with age in lake trout.

Data obtained from the half-life study (Figure 29) show that the Hg is removed from mosquitofish rapidly when the animals are transferred to clean water. The best estimate of the biological half life, based on the data, is 13.5 days. ($\text{C.I.}_{95\%}$ = 9.9-23.1) based on a least squares fit assuming first order kinetics. This half-life, obtained from fish that had been in a contaminated environment their entire lives, is considerably lower than the 60-day half life reported by Huckabee and Blaylock (1973) for mosquitofish contaminated by a short-term direct exposure. Schoper (1974) calculated a half-life of 45 days for Hg in mosquitofish but her data are limited and she presents no confidence interval for her estimate.

BIOLOGICAL RESPONSES

Introduction

During the 41-month project period, the biological communities that developed in the artificial streams

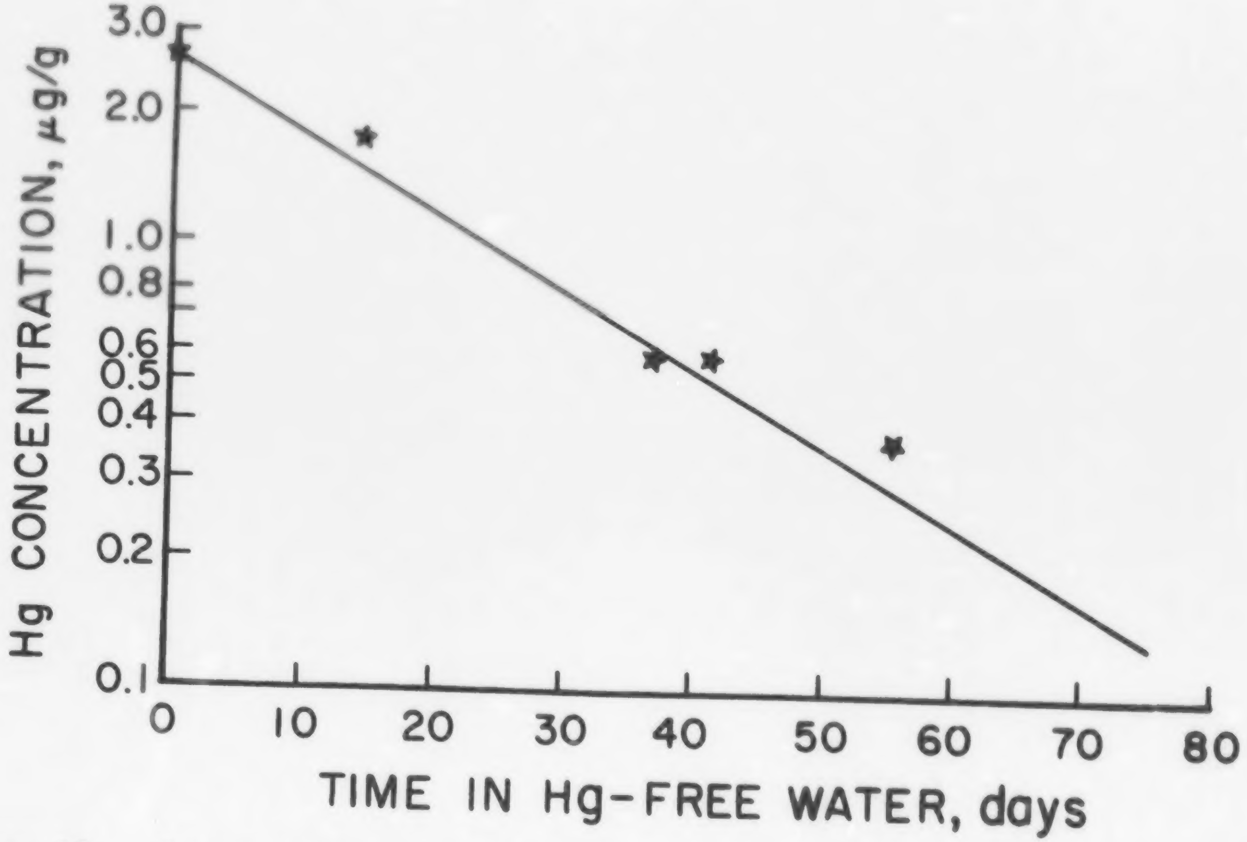

FIGURE 29. CHANGE IN MERCURY CONCENTRATIONS IN MOSQUITOFISH (*GAMBUSIA AFFINIS*) MOVED FROM A CONTAMINATED AREA TO CLEAR WATER. EACH POINT IS BASED ON 10 ANALYSES, EACH OF AN INDIVIDUAL FISH.

were undergoing continuous changes and no equilibrium systems evolved. There was a continual downstream expansion of the macrophyte populations, a constant rearrangement of insect and algal populations, and a continuous influx of new plant and animal species into the channels. It is within this constantly changing pattern that the effects of Hg must be considered. In the statistical analyses of the data, time-treatment interactions often appeared as significant factors in comparisons between treatments.

In the initial planning, it was assumed that the only factor that would vary among the six channels would be the concentration of Hg^{++} in the water. There were several deviations from this ideal situation, however, resulting from the physical structure and positioning of the channels. These included: (1) differential inputs of seed material resulting from the orientation of the channels with respect to the prevailing winds, (2) differential disturbances by vertebrates, including mammals and amphibians, which were largely confined to the outermost channels, and (3) the input of dissimilar water qualities which was a result of the different flow rates through the limestone water treatment systems made necessary by the development of major leaks in several of the head pools. These factors contributed greatly to the variability of the data and decreased the sensitivity of the statistical procedures to reject a null hypothesis of "no effect of Hg" on the various biological parameters studied. Nevertheless, there were effects observed that can be related to the input of Hg.

Ferens (1974) summarizes the data regarding the effects of Hg on the benthic and periphytic communities in the channels prior to the dose increases in August, 1973. Even at concentrations of 0.01 and 1.0 μg/l, levels not uncommon in natural waters (Wershaw, 1970; Jenne, 1972), she found small but significant reductions in algal number, biomass, and diversity, although the number of species affected was small, and there was no indication that these effects were transferred to consumer trophic levels.

Taxonomy

Taxonomic efforts were concentrated on the algae, vascular plants, and insects in the channels. Early in the study, for the identification of algae, a number of samples were sent to Dr. L. A. Whitford at the North Carolina State

University. As new species were found, they were identified by keys and descriptions presented in Huber-Pestalozzi (1938, 1941, 1955), Prescott (1962, 1970), Ralfs (1848), Smith (1950), Tiffany and Britton (1952), and Whitford and Schumacher (1969). Vascular plants were identified by keys in Radford et al. (1968). The identity of the dominant plant, Juncus diffusissimus was confirmed by Dr. J. Coffee of Queens College, Charlotte, N. C. Most of the insects were identified as to genus by reference to keys in Pennak (1953) and Edmondson (1959). Certain dipterans were further identified by Beck (1968) and Mason (1973) and vertebrates were keyed with Blair et al. (1957).

A complete species list of identified channel biota is presented in the Appendix. In addition to these organisms, a variety of bacteria, fungi, protozoa, nematodes, rotifers, tardigrads, oligochaetes, cladocerans, ostracods, copepods, and aquatic mites was also observed during the study. This assemblage of organisms that colonized the streams, including at least 50 species of algae, 5 species of vascular plants, and 61 species of insects, approached a natural ecosystem in complexity and possibilities of interaction. In their review of artificial stream research, Warren and Davis (1971) stress the relationship between the complexity of artificial stream systems and the conclusions that can be extrapolated from them to natural systems. Because of the duration of the Hg study, the complexity of the biological communities that evolved, and the similarity of the water to natural streams in this region, data obtained from the artificial streams should be directly applicable to soft-water streams of the southeastern United States and in a more general way to all aquatic systems.

During the study, with the exception of amphibians, no attempt was made to limit recruitment of new species into the channels. Frog egg masses were removed because these were found only in the outermost pairs of channels. When it became obvious that some eggs had been missed (September, 1973), an attempt was made to place an equal number of eggs (Rana pipiens) in each channel. Based on later observations, it appears that few, if any, of the eggs placed in Hg-treated channels hatched. This was probably a direct result of the Hg treatment. Birge et al. (1974) found that levels of 10 µg/l of inorganic Hg were 100% effective in preventing the development of Rana pipiens embryos when applied during the cleavage of blastula stage. During the period after the Hg inputs were stopped, amphibian eggs successfully completed development in all channels and an unknown number of tadpoles of at least three species (see Appendix) were observed.

At the end of the project, an attempt was made to quantitatively remove all vertebrates and larger invertebrates from the channels. The results of these efforts are summarized in Table 4. These results were somewhat unexpected in that the great differences that existed between channels with respect to fish, snail and tadpole densities were never obvious from the routine sampling programs. There is no question but that the density differences in these animals, which appear to be totally unrelated to Hg treatment, affected other channel components. These effects contribute to the variability of the data and make it even more difficult to determine responses due to Hg treatment.

Algae

All of the algae identified in the artificial streams during the course of this study could be described as tychoplanktonic, attached or benthic algae characteristic of soft, acidic waters of low-current velocity. The algal community was clearly dominated by members of Chlorophyta (green algae) with desmids and filamentous forms the most abundant. Diatoms were very poorly represented with only four species observed and only two of these occurring regularly. The diatoms were not limited in the streams by a lack of silicon in the water (Table 1), as the level observed was well above the silicon concentration in nearby diatom-dominated systems, but rather from a combination of adverse environmental parameters such as the lack of shading, the very slow current velocity, and the year-round water temperatures above 20°C. The Cyanophyta or blue-greens were never dominant either, but one species, *Merismopedia punctata*, was present in almost every sample taken. Filtering of large volumes of water from the channels indicated that truly planktonic algae were not common although *Dictyosphaerium pulchellum* was occasionally seen.

Community Responses-

Of the various substrates in the streams that were regularly sampled, the walls contained the oldest and least-disturbed communities of periphyton. Figure 30 summarizes the biomass results obtained from the PVC strips during the first part of the study. Figure 31 shows the biomass levels of the samples from the stream walls during the latter part of study. The apparent abrupt increase in wall biomass between October, 1973, and January, 1974, with respect to the 5-μg/l channels may have been a result of the change in sampling technique rather than a real increase

Table 4. ORGANISMS COLLECTED FROM THE ARITIFICIAL STREAMS, FEBRUARY 11-16, 1975

Organism (* indicates a new species on this date)	Channel number					
	1	4	2	5	3	6
	Control		1.0 µg/l		5.0 µg/l	
Odonata:						
Argia sp.	430	637	545	591	465	53
Ischnura sp.	7	131	101	318	314	296
Erythrodiplax miniscula	162	187	858	451	297	47
Pantala hymenea	10	3		4		
**Ladona deplanta*	3	12		14		
**Celithemis fasciata*	11	110	12	89	84	8
**Tetragoneuria semiaquea*	7			1		
**Plathemis lydia*	1					
**Pacydiplax longipennis*					2	
Ephemeroptera:						
Caenis sp.	26	14	18	68	15	
Callibaetis sp.		4	4	3	5	
Hemiptera:						
**Ranatra* sp.		2				
Pelocoris femoratus	21	14	31	20	9	2
Hesperocorixa spp.	44	40	44	46	22	154
Megaloptera:						
**Chauliodes* sp.	2		14			
Diptera:						
Tanypodinae	10		3	4		
Chironominae	53	4	186	6	140	
pupae	6	1	12			
**Tipula* sp.	6		27	3	1	
Helius sp.		2	2	1	2	
Dasyhelea sp.	1		6	1	2	

Table 4 (continued)

	Channel Number					
	1	4	2	5	3	6
Organism	Control		1.0 µg/l		5.0 µg/l	
Lepidoptera:						
Parapoynx sp.	3	13	8	19	34	3
*_Synclita_ sp.	1		32	23	2	3
Coleoptera:						
Berosus spp.	4	9	18	33	24	38
Coptotomus sp.	2	6	6	2	7	6
*_Tropisternus_ #1	2	1		1		4
*_Tropisternus_ #2	1		1			9
Hydocanthus sp.	44	13	62	50	11	1
Hydroporus sp.		1	9	1	15	3
*_Agabus_ #1	1					
*_Agabus_ #2			1			
*_Enochrus_ #1			1	1		
*_Enochrus_ #2					1	
Laccophilus sp.					1	1
Others:						
snails (_Physa_ and _Lymnea_)		405	4	262		1077
Rana spp.	30	30	284	489	129	629
Gambusia affinis	167	246	217	172	148	76
Total Insects	858	1204	2001	1750	1453	628
Insects/meter^2	15.4	21.6	35.9	31.4	26.1	11.3
# insect species	25	20	24	24	21	15
$\bar{H}$ - insects	2.58	2.31	2.56	2.70	2.73	2.28
E - insects	0.56	0.53	0.56	0.59	0.62	0.58
Grand Total	1055	1886	2506	2673	1730	2410
# species	27	23	27	27	23	18
$\bar{H}$	2.91	2.84	2.98	3.20	3.08	2.28
E	0.61	0.63	0.63	0.67	0.68	0.55

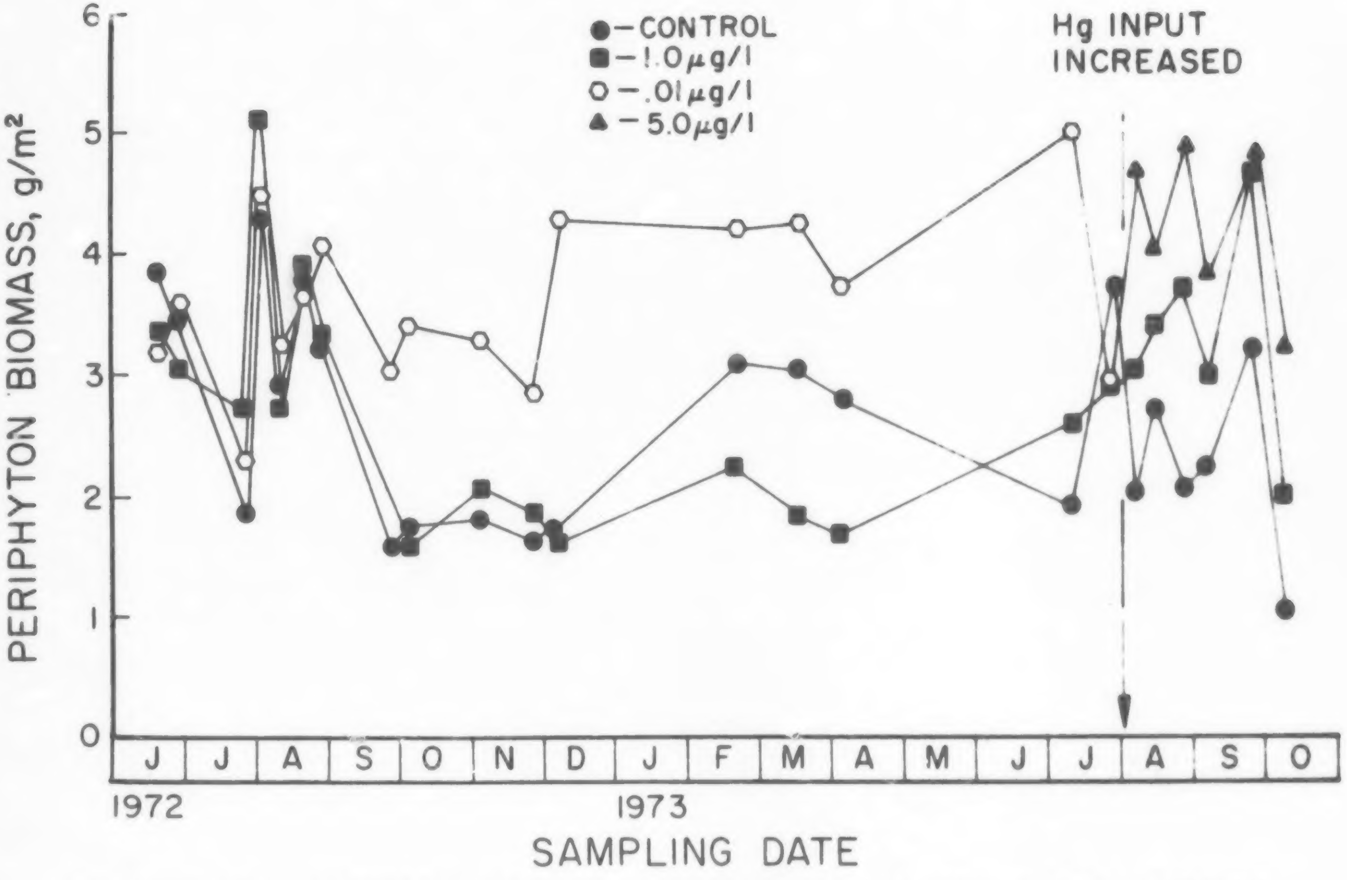

FIGURE 30. BIOMASS OF PERIPHYTON SCRAPED FROM PVC STRIPS SUSPENDED IN THE CHANNELS.

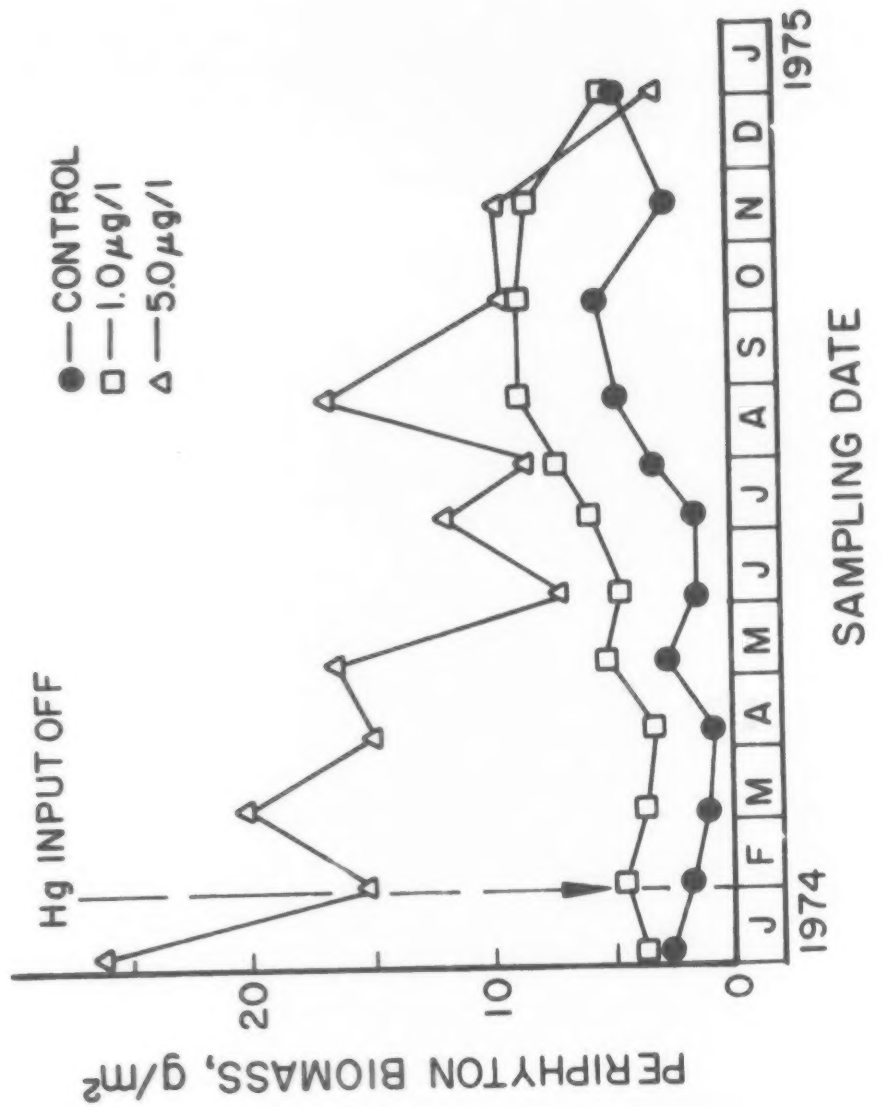

FIGURE 31. BIOMASS OF PERIPHYTON SCRAPED FROM CHANNEL WALLS.

during this time interval. There was a certain amount of loss from the PVC strips when they were removed from the channels and these losses were relatively greater when the growth was heavy, as it was in the 5.0-μg/l channels.

The periphytic biomass in all channels fluctuated similarly during the summer of 1972 probably due to environmental changes, but in September, channels 3 and 6 (0.01 μg/l at that time) did not follow the biomass reductions that occurred in the other streams, indicating a possible stimulation in growth at low-Hg levels. After the Hg levels in channels 3 and 6 were increased to 5.0 μg/l in August, 1973, the walls of these channels continued to support higher periphytic biomasses than in the other channels. Figure 31 illustrates a direct correlation between the Hg concentration in the streams and the standing crops of periphyton inhabiting the walls, with a subsequent equalization in biomass levels after the input was shut off. A stimulation of algal growth at a Hg concentration as high as 50 μg/l has been reported (Matsui and Gloyna, 1972).

Figure 32 shows that the algal component of the wall periphytic communities, as summarized by total cell volumes, followed the same general pattern as shown in the biomass data. In October, 1974, the total algal volume was greater in the control channels than in the treated, although this pattern is not repeated in the biomass data of the same date. This indicates that, at least at that sampling time, the non-algal component of the periphyton, either living or dead, made up a greater percentage of the organic matter on the walls of the Hg-treated channels.

Figure 33 summarizes the calculated diversity and evenness values for these algal components of the periphytic communities in time. The control and low-level streams were quite similar with respect to these parameters (as they were in biomass and algal volume), and the high-level streams initially had decreased diversity and evenness values. An analysis of variance (ANOVA) of the data collected while Hg was still being input to the channels showed there was a significant treatment effect ($p < .05$) on $\overline{H}$, and highly significant effects ($p < .01$) on E and cell volume.

Although biomass values of periphyton collected from vertical plexiglass plates were quite variable (Figure 34), certain trends are evident. During 1972, the biomass levels on this substrate had slightly lower values than were found on the PVC strips. Channels 3 and 6 again diverged from the other channels in the fall of 1972 with

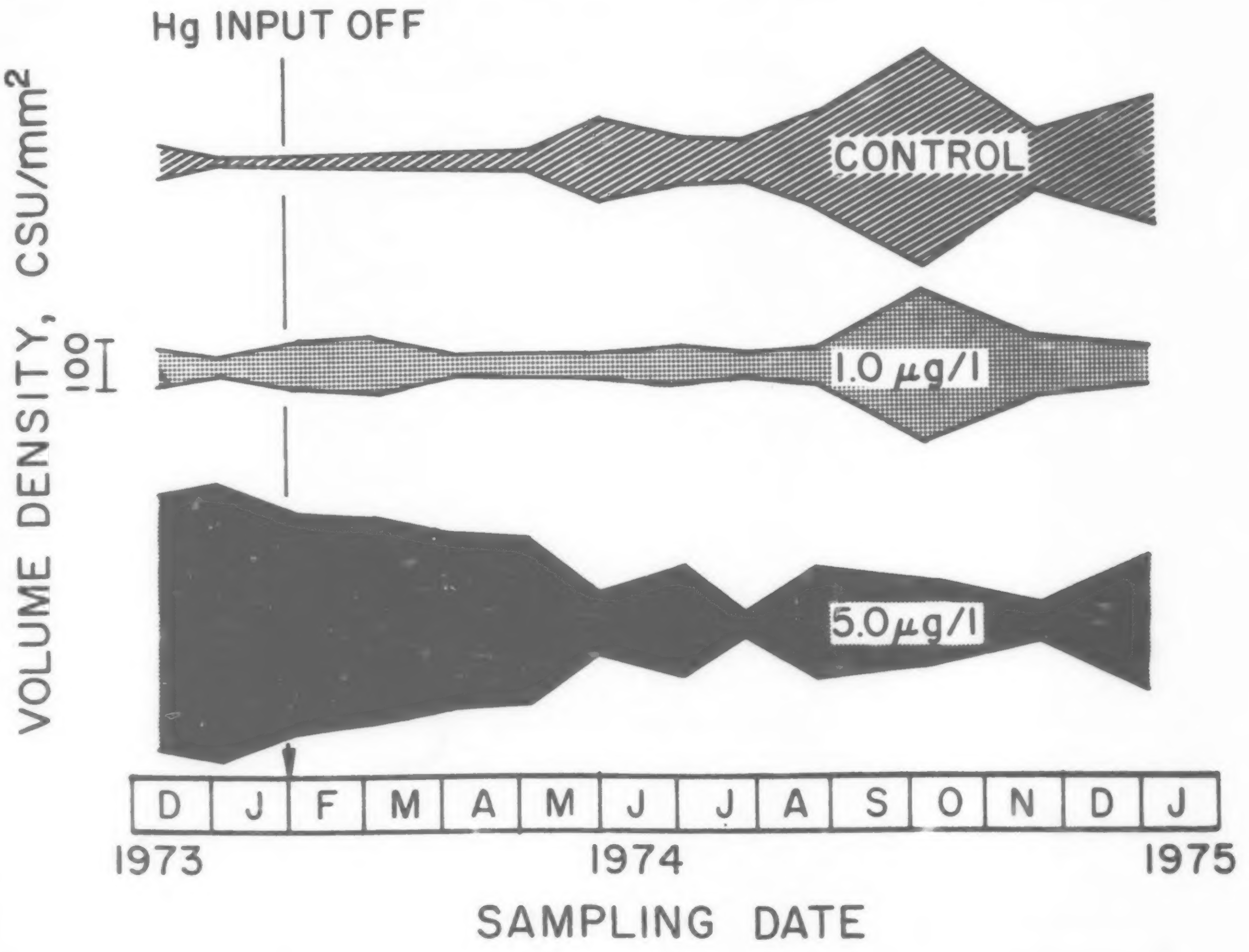

FIGURE 32. CALCULATED VOLUME OF PERIPHYTIC ALGAE SCRAPED FROM THE CHANNEL WALLS.

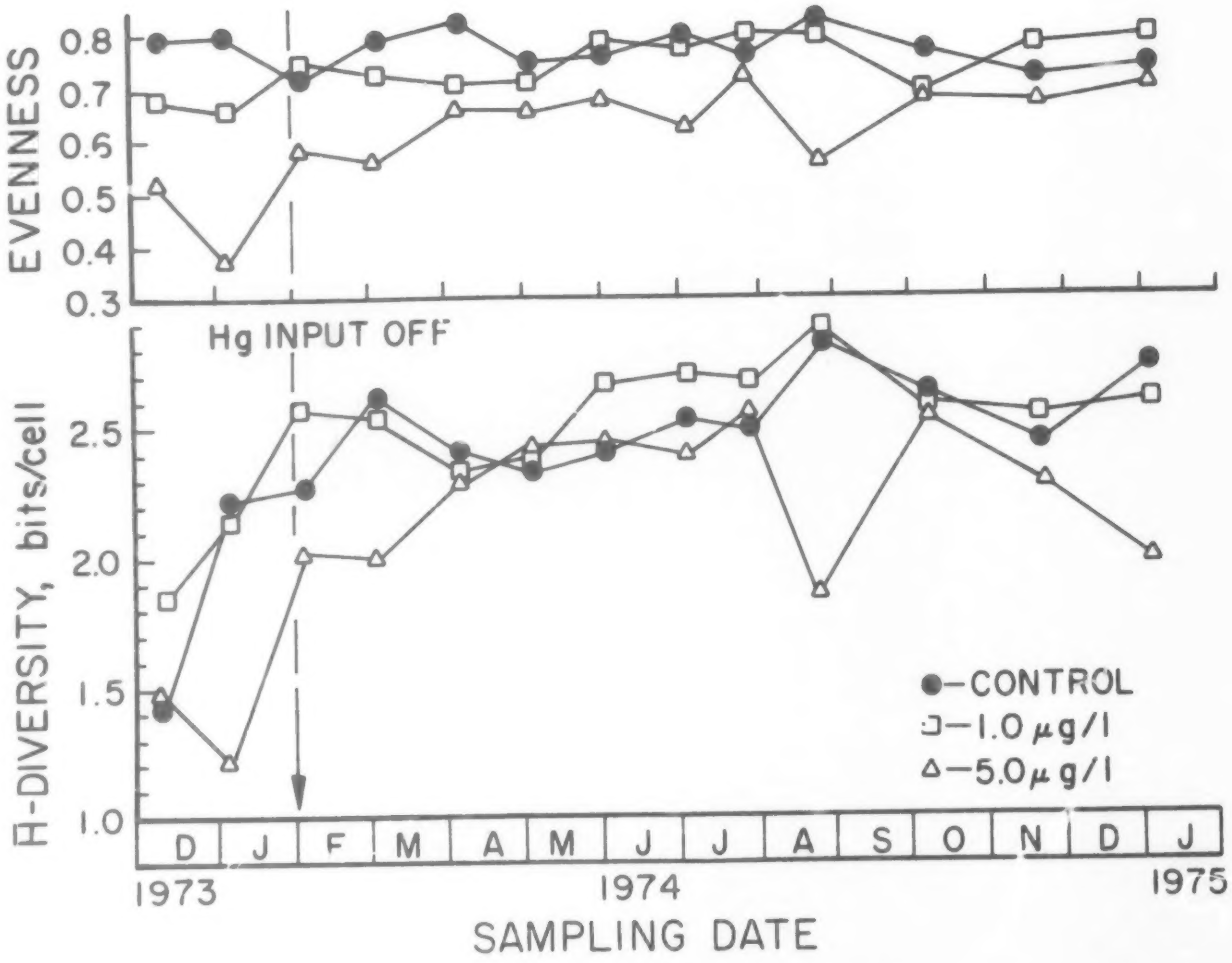

FIGURE 33. DIVERSITY AND EVENNESS OF PERIPHYTIC ALGAE COLLECTED FROM THE CHANNEL WALLS.

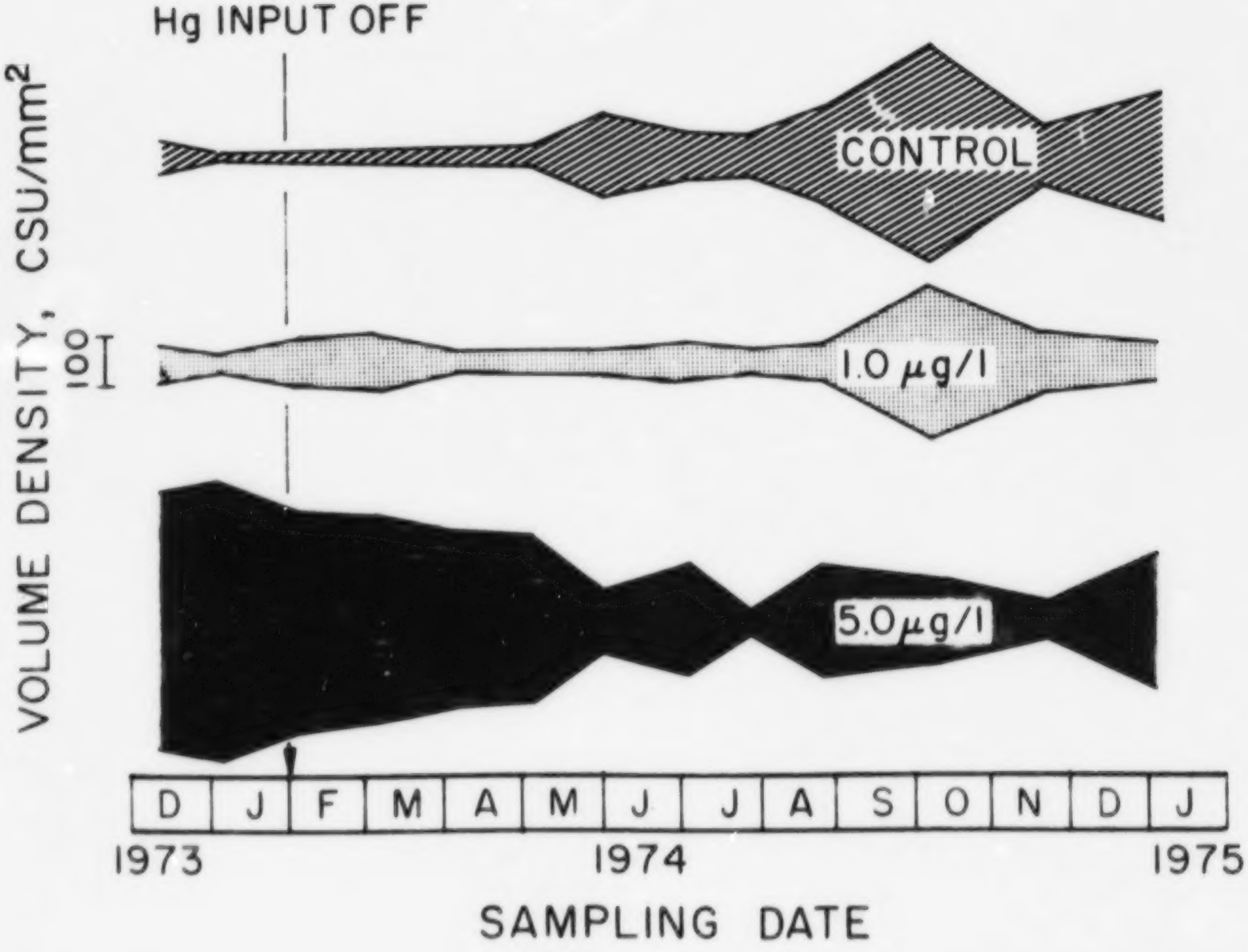

FIGURE 32. CALCULATED VOLUME OF PERIPHYTIC ALGAE SCRAPED FROM THE CHANNEL WALLS.

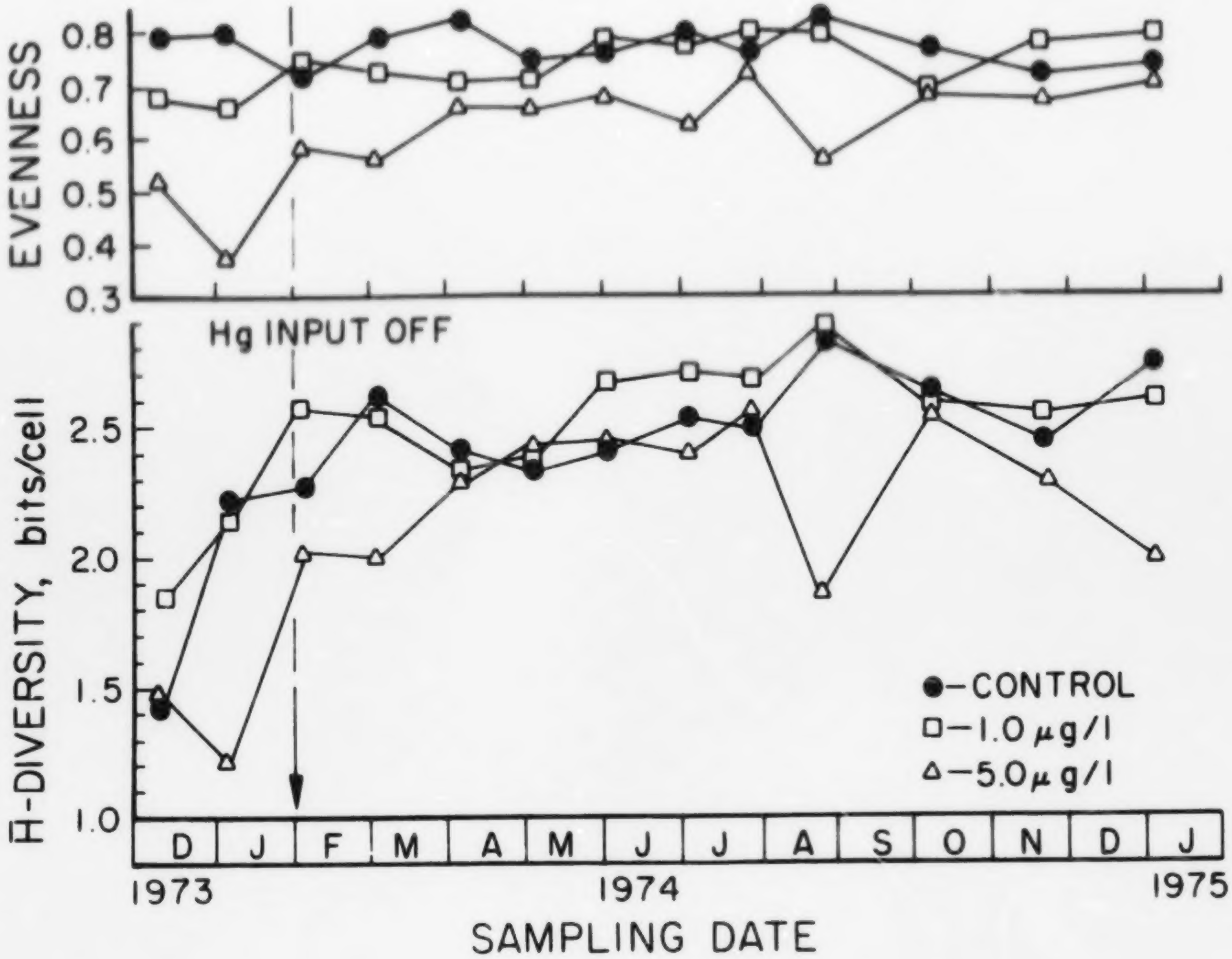

Figure 33. Diversity and evenness of periphytic algae collected from the channel walls.

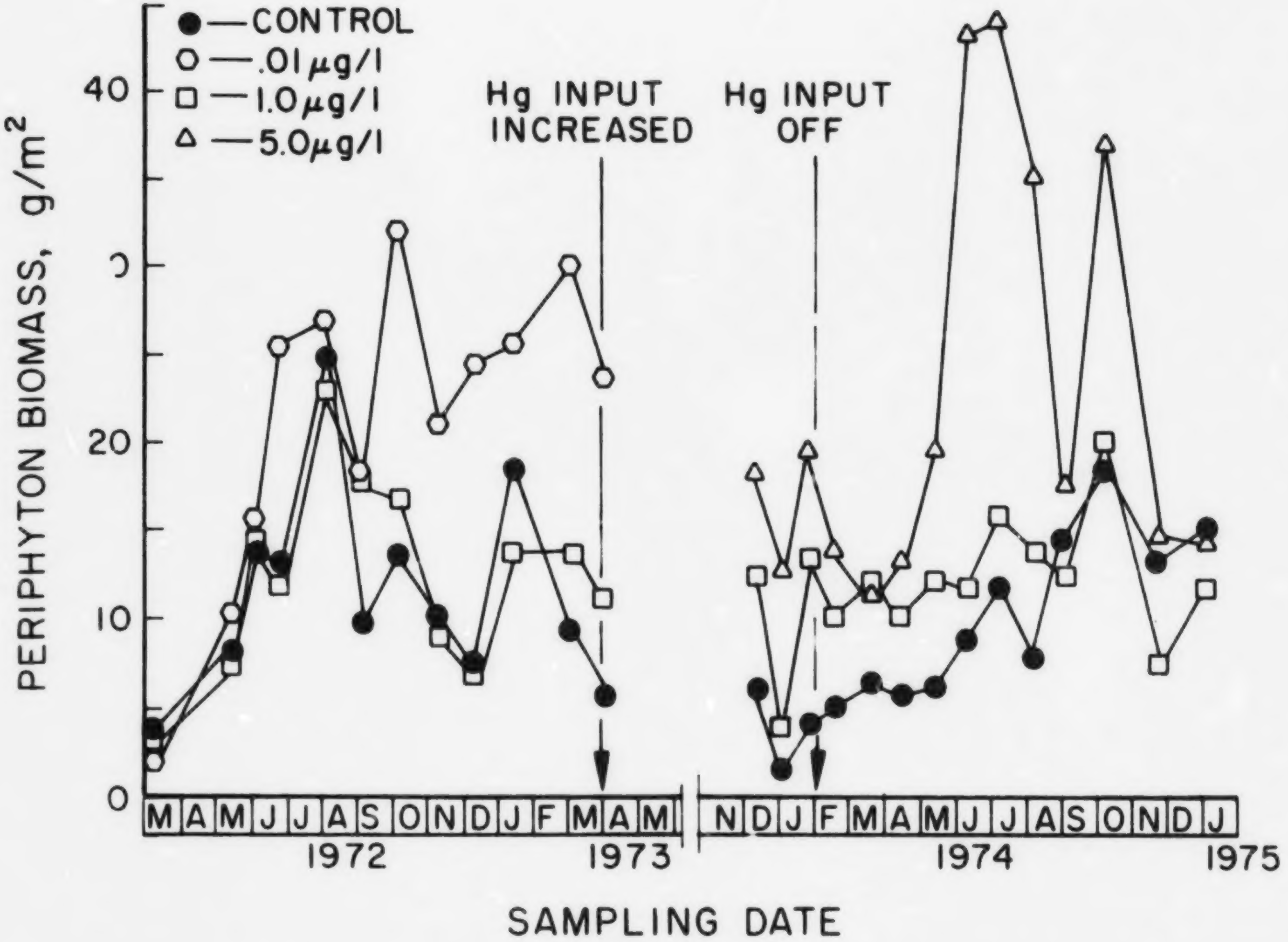

FIGURE 34. BIOMASS OF PERIPHYTON SCRAPED FROM VERTICAL PLEXIGLASS PLATES.

higher biomass values. This pattern continued after the Hg input levels were increased in August, 1973, until the winter of 1974, 11 months after the Hg was shut off. At the end of the project, all channels were similar. The graph summarizing algal cell volumes for the plexiglass plates (Figure 35) shows that the increase in biomass observed in streams 3 and 6 was partially the result of increased algal growth at that time. Figure 36 shows that algal diversity and evenness of periphytic samples removed from plexiglass plates were lowered by Hg treatment during the period Hg was input to the streams. These differences were significant (ANOVA, $p < 0.01$).

The results obtained from the first two glass slide experiments are summarized in Figures 37 and 38 in terms of the calculated algal volumes. These two experiments were carried out while Hg was still being added to the channels. Both graphs demonstrate the significant delay in the colonization of microscope slides by algae in the 5.0-μg/l channels and the similarity between the control and 1.0-μg/l channels.

In both experiments, the diversity and evenness of the algal component of the periphytic communities were significantly (ANOVA, $p < 0.01$) affected by the treatment with the highest diversities in the high-level streams. On a small number of slides from these experiments examined after 40 and 80 days of colonization, the trend towards lowered algal biomass with higher Hg exposure had disappeared and even reversed, with the highest algal densities occurring on the slides from the 5.0-μg/l channels. The observed increases in algal biomass in the high-level streams were not accompanied with any decreases in diversity or evenness.

The initial retardation of algal growth on microscope slides placed in the 5.0-μg/l channels disappeared in the glass slide experiments carried out after the inputs of Hg were stopped. Figure 39 illustrates algal volume changes on slides placed in the channels one day after the Hg was turned off. There were no significant treatment effects found for these data with respect to cell volume, diversity or evenness (ANOVA, $p > 0.05$). Figure 40 summarizes the data from a glass slide colonization experiment started three months after inputs were stopped. While the former control and low-level channels were similar in cell volumes and diversities throughout the study, the former high-level channels developed larger cell volumes and lower diversity values.

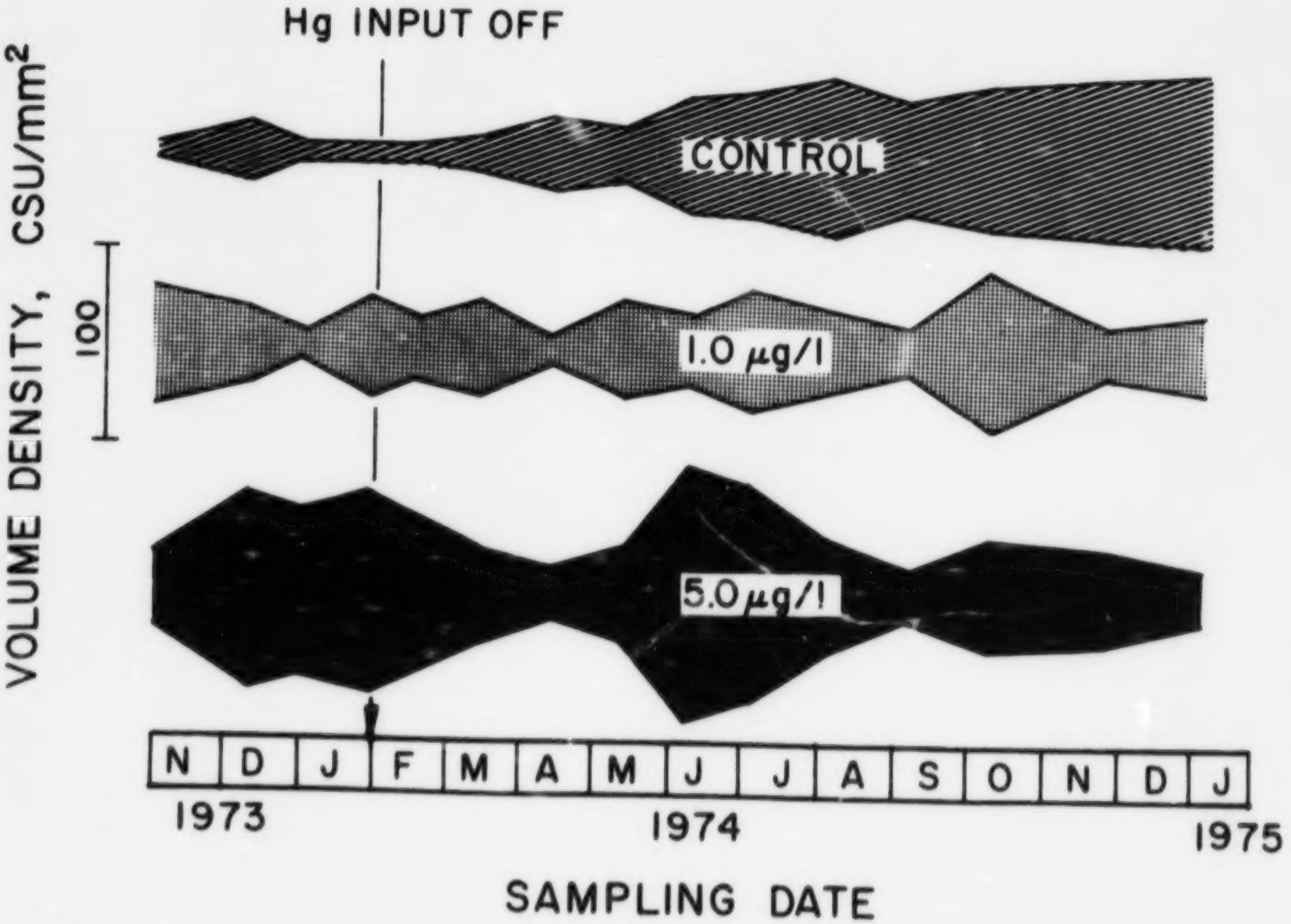

FIGURE 35. CALCULATED VOLUME OF PERIPHYTIC ALGAE SCRAPED FROM VERTICAL PLEXIGLASS PLATES.

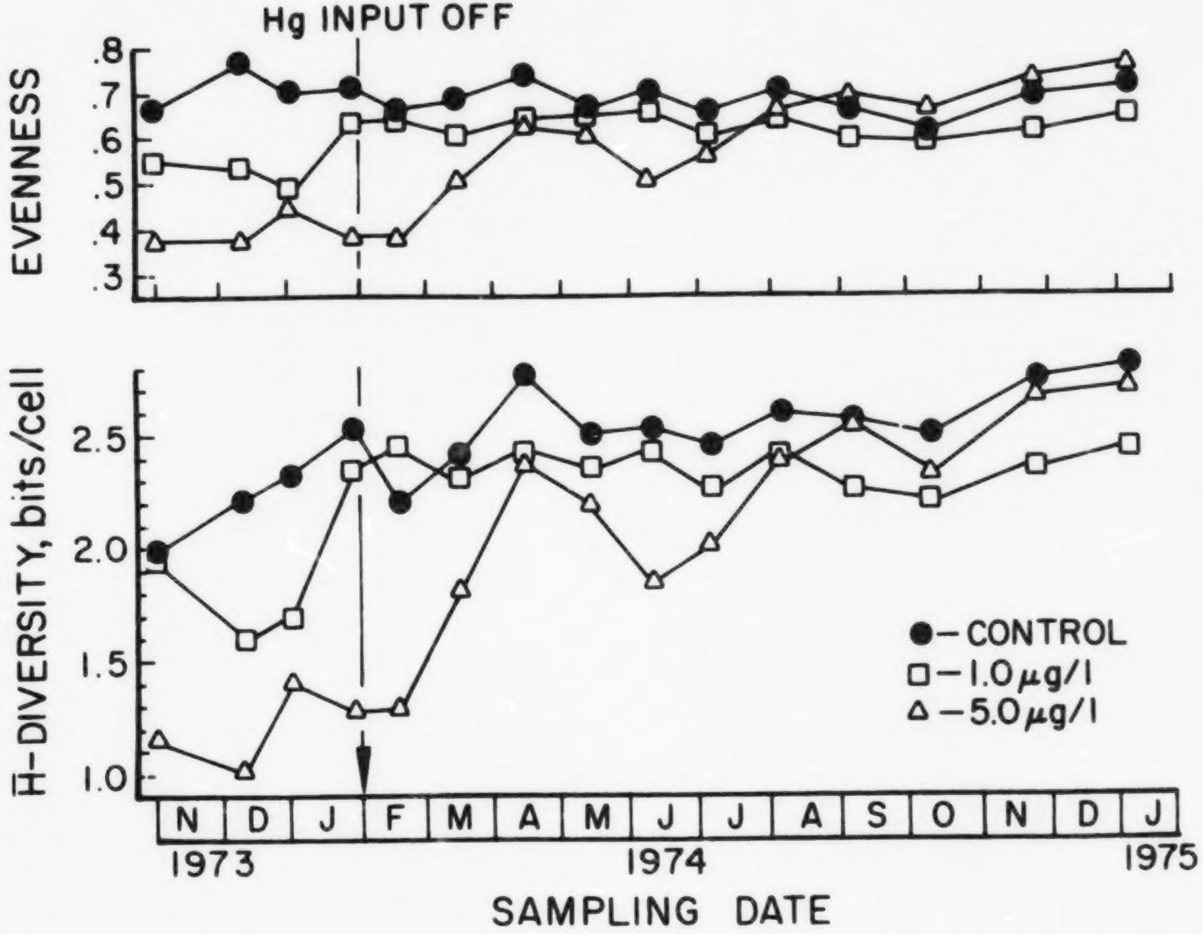

FIGURE 36. DIVERSITY AND EVENNESS OF PERIPHYTIC ALGAE COLLECTED FROM VERTICAL PLEXIGLASS PLATES.

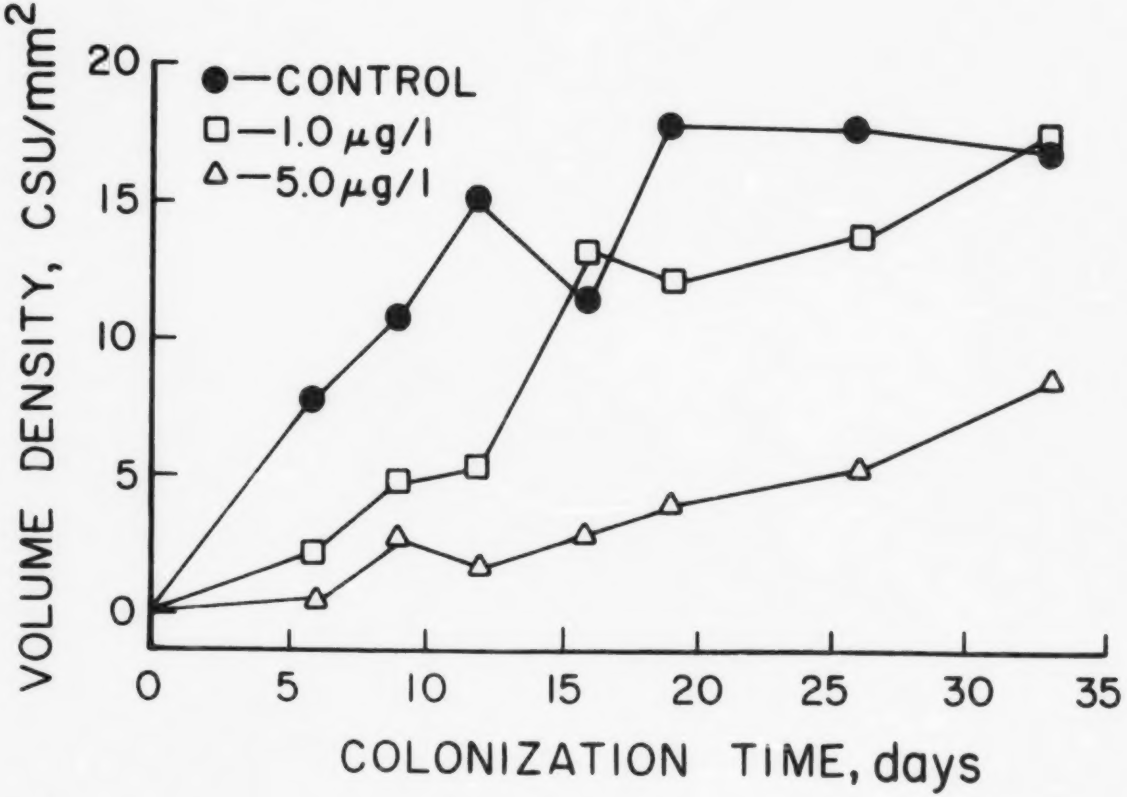

FIGURE 37. CALCULATED VOLUME OF ALGAE COLONIZING MICROSCOPE SLIDES DURING MERCURY INPUTS (STARTED OCTOBER 31, 1973).

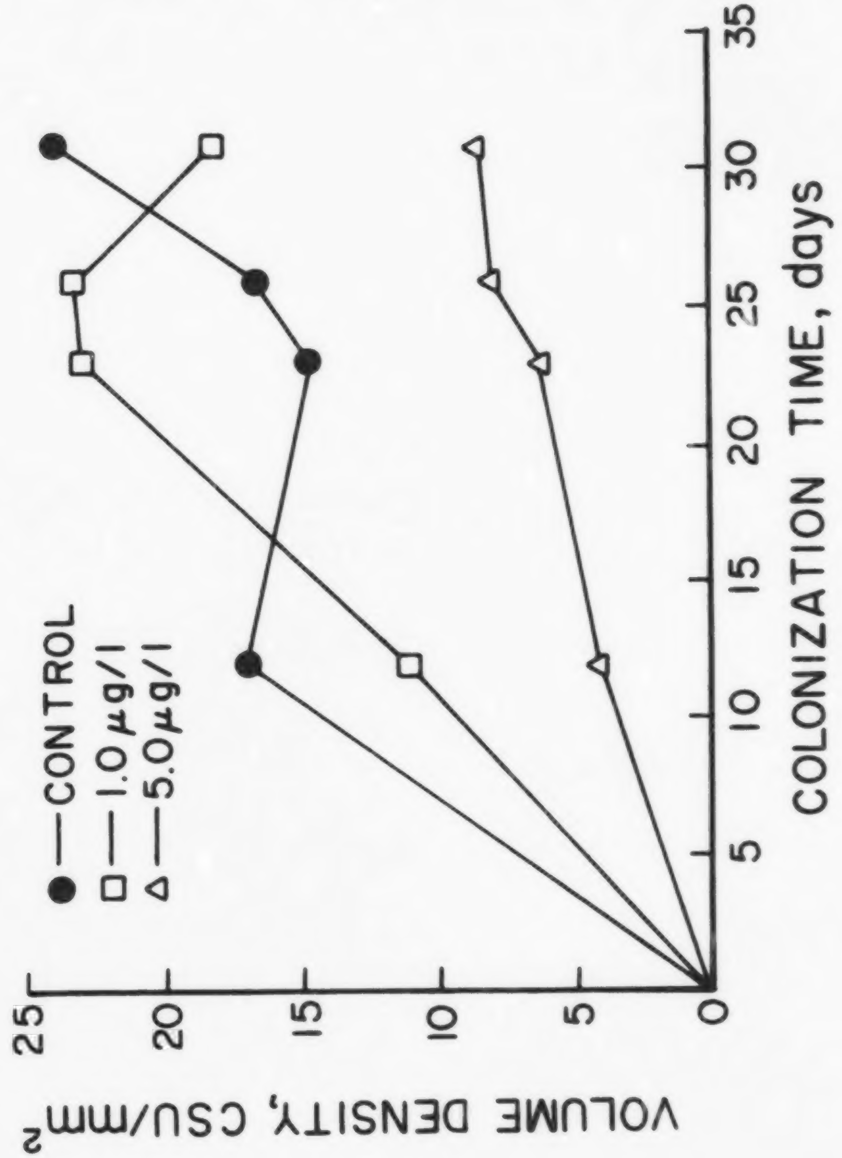

FIGURE 38. CALCULATED VOLUME OF ALGAE COLONIZING MICROSCOPE SLIDES DURING MERCURY INPUTS (STARTED DECEMBER 7, 1973).

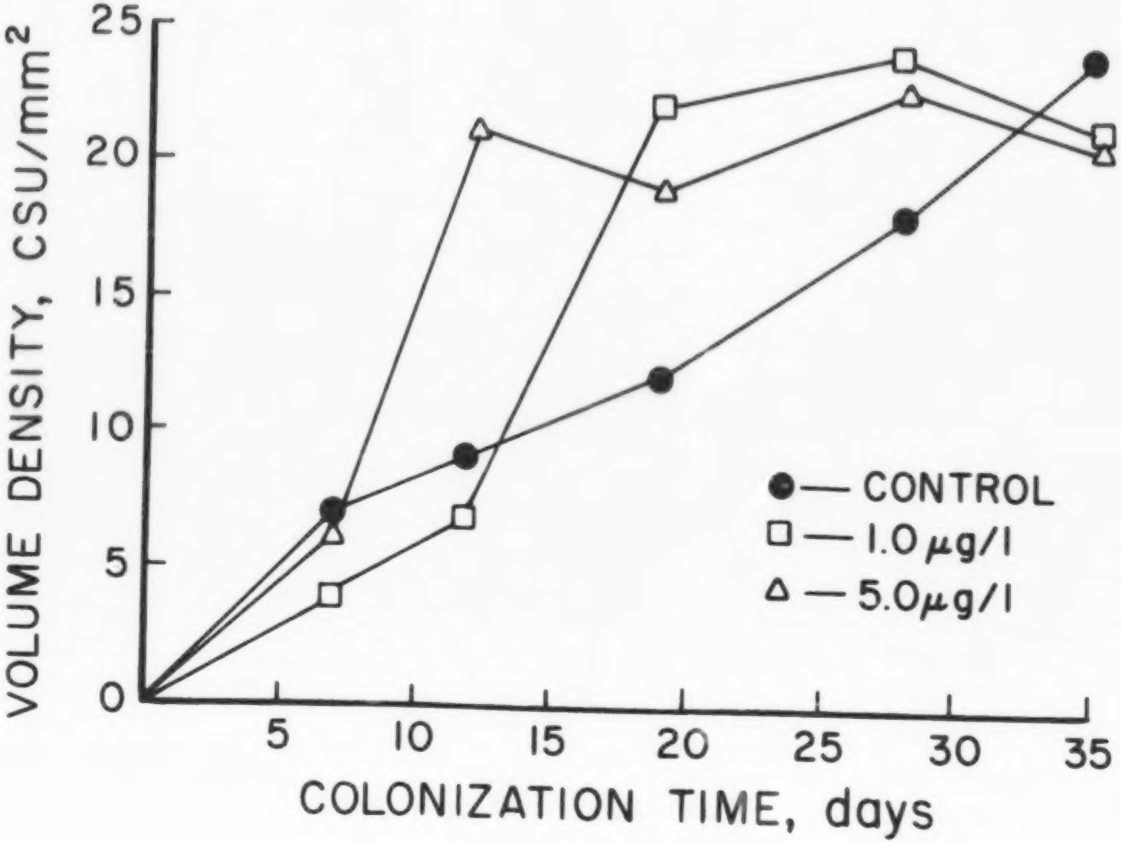

FIGURE 39. CALCULATED VOLUME OF ALGAE COLONIZING MICROSCOPE SLIDES AFTER MERCURY INPUTS WERE STOPPED (STARTED JANUARY 30, 1974).

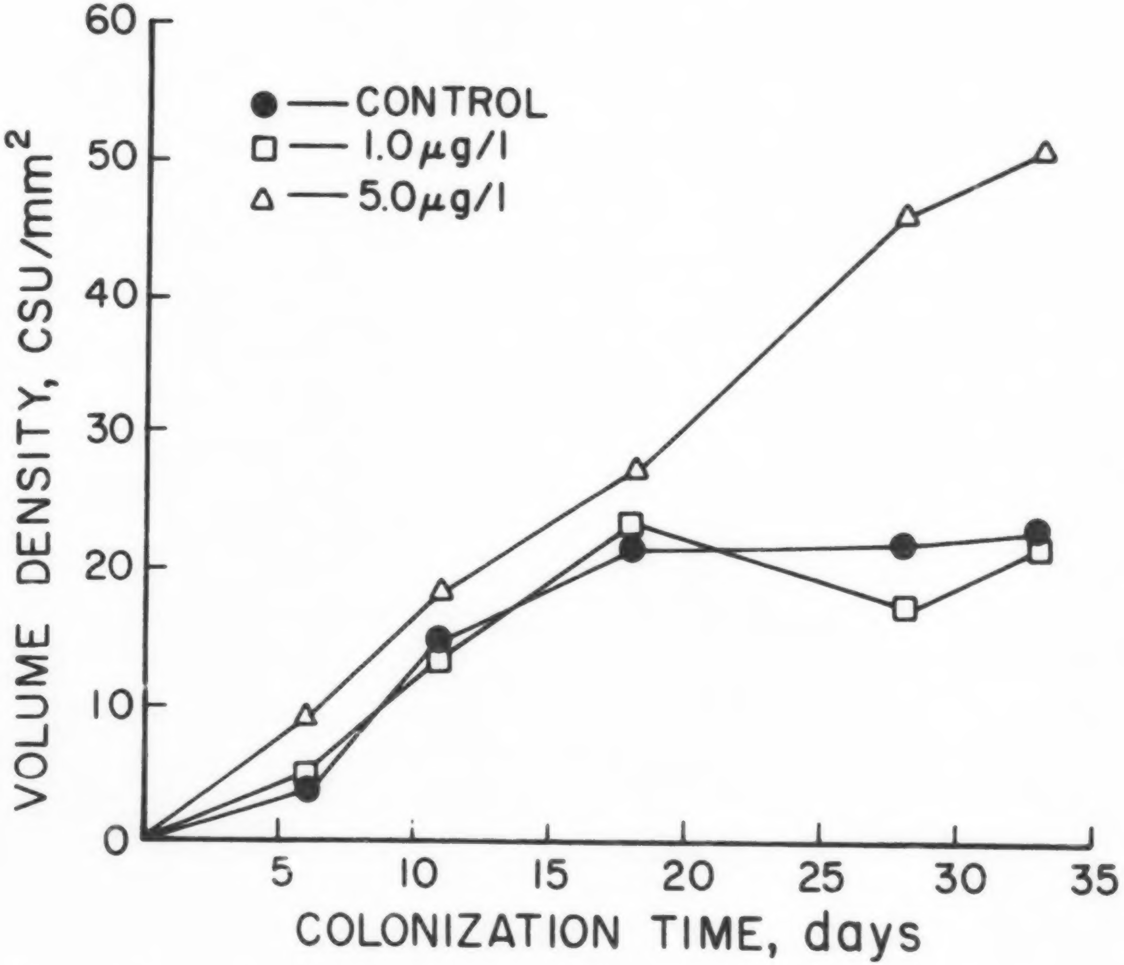

FIGURE 40. CALCULATED VOLUME OF ALGAE COLONIZING MICROSCOPE SLIDES AFTER MERCURY INPUTS WERE STOPPED (STARTED APRIL 25, 1975).

The final glass slide colonization experiment was begun in October, 1974, nine months after Hg inputs were stopped. During this experiment, the control and former 5.0 μg/l channels were very similar, and the algal volumes in the former 1.0-μg/l channels were consistently lower. There were no significant differences between the treatments with respect to diversity and evenness.

In August, 1974, experiments were initiated in the continuous flow chambers to check some of the results observed in the channels. In the first of three experiments with algae, mixed cultures containing *Oedogonium* sp., *Stigeoclonium elongatum*, *Chlamydomonas* sp., *Oscillatoria geminata*, and *Microspora quadrata*, species isolated from the artificial streams, were used as seeding material. Three boxes received a Hg input of 5.0 μg/l and three were used as controls. Colonization on these glass slides was observed for 10 days and was found to be dominated by the *Oscillatoria geminata* and *Chlamydomonas* sp. with very few *S. elongatum* or *Oedogonium* sp. and no *M. quadrata* observed on the slides. After 10 days, the mean number of cells/mm^2 was 1256 ± 357 ($\bar{X}$ ± SE) for the controls and 10.0 ± 3.6 ($\bar{X}$ ± SE) for the treated chambers. The colonization of both *O. geminata* and *Chlamydomonas* sp. was inhibited in the Hg-treated chambers. The limited number of *Chlamydomonas* sp. that were observed were immobile in palmelloid groups that did not stir while the slides were being counted. When the Hg was cut off in these boxes, these cells were observed to develop flagella and start swimming as had been continuously observed on slides from the control chambers. Although the *M. quadrata* did not develop on the glass slides in any of the chambers, lush growth of this filamentous green algae did develop in the outflow holes of the control chambers, while very little growth occurred in this position in the Hg chambers.

In a single-species experiment with *S. elongatum*, very little growth was obtained over a 25-day period in the treated chambers while this and other contaminating species grew profusely in the control chambers. After the Hg input was discontinued, five clean slides were placed in each chamber. The colonization of *S. elongatum* was observed and found not to differ between the chambers. These experiments indicated a very pronounced retardation of growth of certain algae at a Hg concentration of 5.0 μg/l, and confirm the trends seen in the artificial streams.

The observations from the artificial substrates and the system presented above can be summarized as follows. While the Hg was input into the 5.0 μg/l channels, there was an initial inhibition of algal growth which disappeared after about 40 days and was replaced by increased cell numbers and periphytic biomasses compared to the controls. After the Hg inputs were shut off, the initial inhibition previously found was nonexistent but the trend towards higher biomasses in the former 5.0-μg/l channels continued for about nine months. The algal population dynamics in the 1.0-μg/l channels showed very little modification as compared to the controls throughout the study.

Individual Responses-

Thirty-one of the algal species listed in the Appendix were found at one time or another in all of the six channels. Although the other species were not observed in all channels, with one exception (*Microthamnion strictissimum*), their absence was not related to Hg treatments. *M. strictissimum* was found only in the four treated channels. The species that appeared to be absent from one or more streams were rare wherever they were found. Two exceptions were *Navicula notha*, a motile diatom that was very abundant in channel 6 but never seen in channel 1, and *Spirogyra* sp., which became very common in the tail area of channel 1 but never was found in channels 3, 5, and 6. No definite reasons can be presented to explain the localized abundances of these algae, but they probably illustrate differences between the biota of the channels caused by a combination of random events and possibly Hg treatments.

Detailed cell counts of algae from the various substrates indicated that certain species were important in creating biomass differences between the treatments. A few species of algae were found to be quite sensitive to the low Hg levels used, and one species exhibited morphological alterations apparently attributable to Hg concentrations.

Oedogonium sp. - This green alga was present throughout the study and, although a few cells which may have been oogonia were observed, it was never observed in a true fruiting condition and identification as to species was not possible. Dr. L. Whitford of N. C. State University tentatively called it *O. reinschii* because of the lack of fruiting filaments and the presence of some diamond-shaped cells he observed. However, continuous examination of this species during the last year of the study indicated that it was smaller (usually 3.5 μm in diameter) than the typical *O. reinschii* (5-11 μm) and fit very closely the description of sterile filaments of *O. pusillum*.

Initial colonization of this species on glass microscope slides was rapid in the control and 1.0 μg/l channels but significantly slower in the 5.0-μg/l channels during the two experiments carried out when Hg was being input. In two of three colonization experiments performed after the Hg input was shut off there was no significant difference between the former treatments. In the third experiment, a significantly greater growth was observed in the former 1.0-μg/l streams.

The occurrence of this species on the long-term substrates is summarized in Figures 41 and 42. This species was much more abundant in the high-level streams than in the low-level streams or in the controls during the input of Hg, but during the year of recovery this dominance disappeared on both the walls and plexiglass plates. No seasonal variation was observed for the populations in the two control channels where this species remained at a density of about 300 cells/mm^2 throughout the year.

These data indicate that the reproductive and vegetative portions of the life cycle of *Oedogonium* sp. were differentially affected by low Hg levels. In spite of the large population densities in the Hg-treated channels, the slower colonization of this species on glass slides indicates that its reproduction was affected by the Hg treatment. Since no fruiting filaments were observed in any of the channels, reproduction was asexual by means of zoospores. The zoospores, or zoospore production, therefore, must have been sensitive to the Hg levels in the water although the growth of vegetative filaments was stimulated.

Stigeoclonium elongatum - This species was found to be very sensitive to Hg. During the first two years, a significant growth stimulation on plexiglass plates was observed in the 0.01-μg/l channels and a slight reduction in abundance was found in the 1.0-μg/l channels as compared to the controls. When the Hg concentrations in channels 3 and 6 were increased to 5.0 μg/l, the numbers of cells of *S. elongatum* were significantly reduced. Averages (cells/mm^2) based on 14 counts from each treatment while Hg was being input were 46 ± 12 ($\bar{X}$ ± SE) for the controls, 25 ± 7 for the 1.0-μg/l and 11 ± 5 for the 5.0-μg/l levels.

Figure 43 summarizes plexiglass plate data from the last year of the project. After the Hg was shut off, all of the streams went through similar seasonal fluctuations with summer maxima lasting for about 4-5 months. During this latter period, *S. elongatum* was much more common in

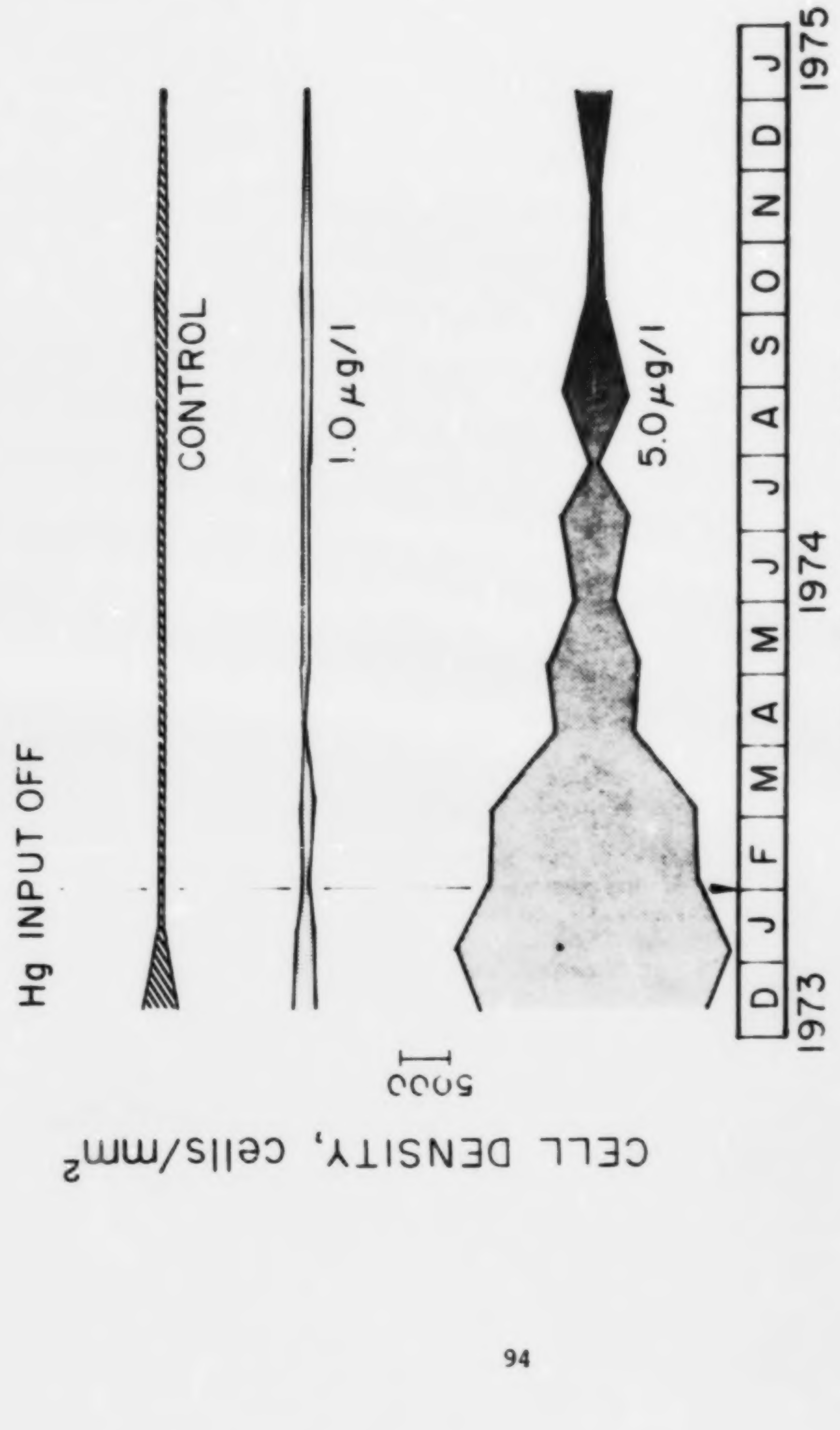

FIGURE 41. DENSITY OF *OEDOGONIUM* SP. ON CHANNEL WALLS BEFORE AND AFTER MERCURY INPUTS WERE STOPPED.

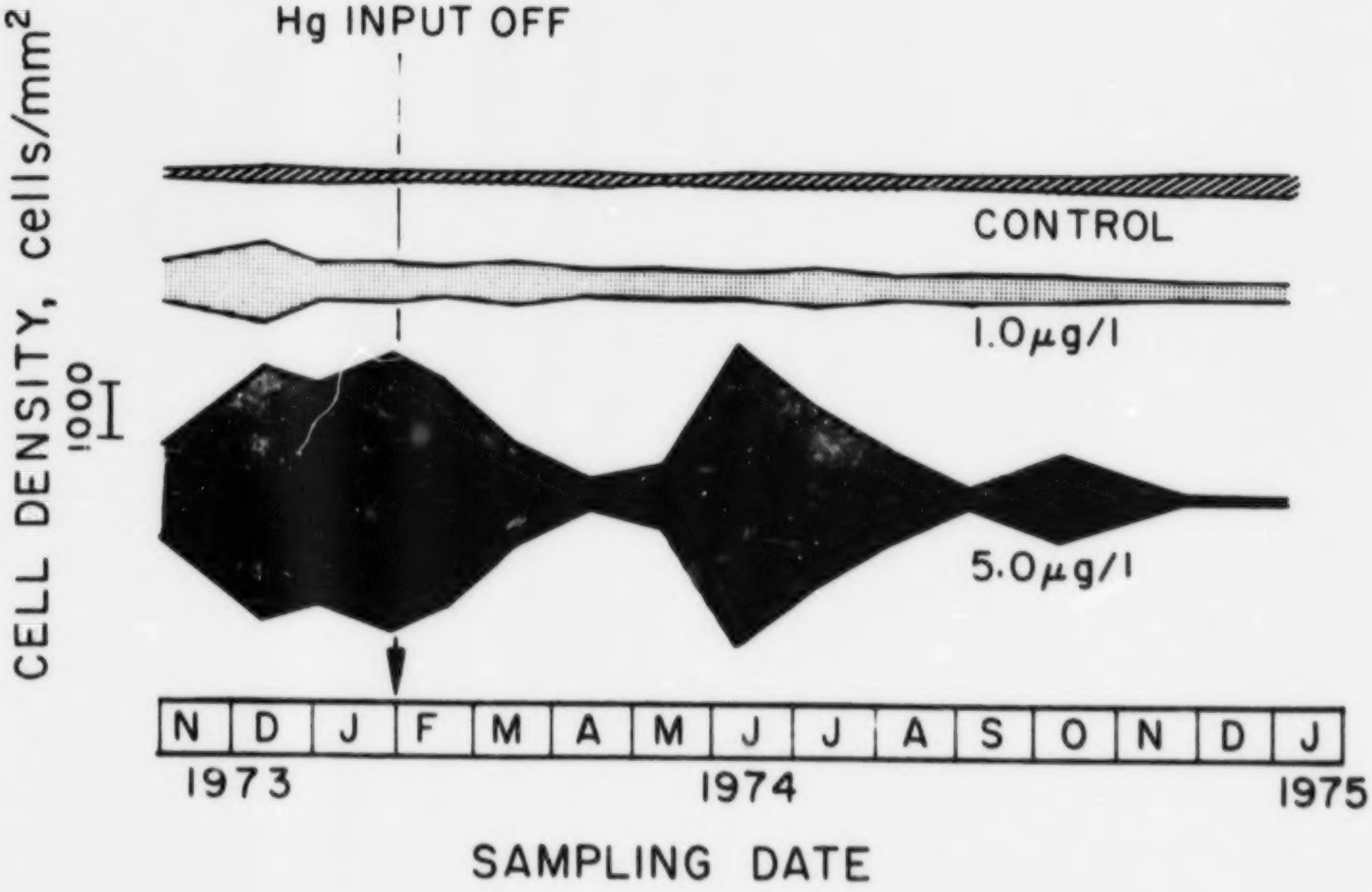

FIGURE 42. DENSITY OF *OEDOGONIUM* SP. ON PLEXIGLASS PLATES BEFORE AND AFTER MERCURY INPUTS WERE STOPPED.

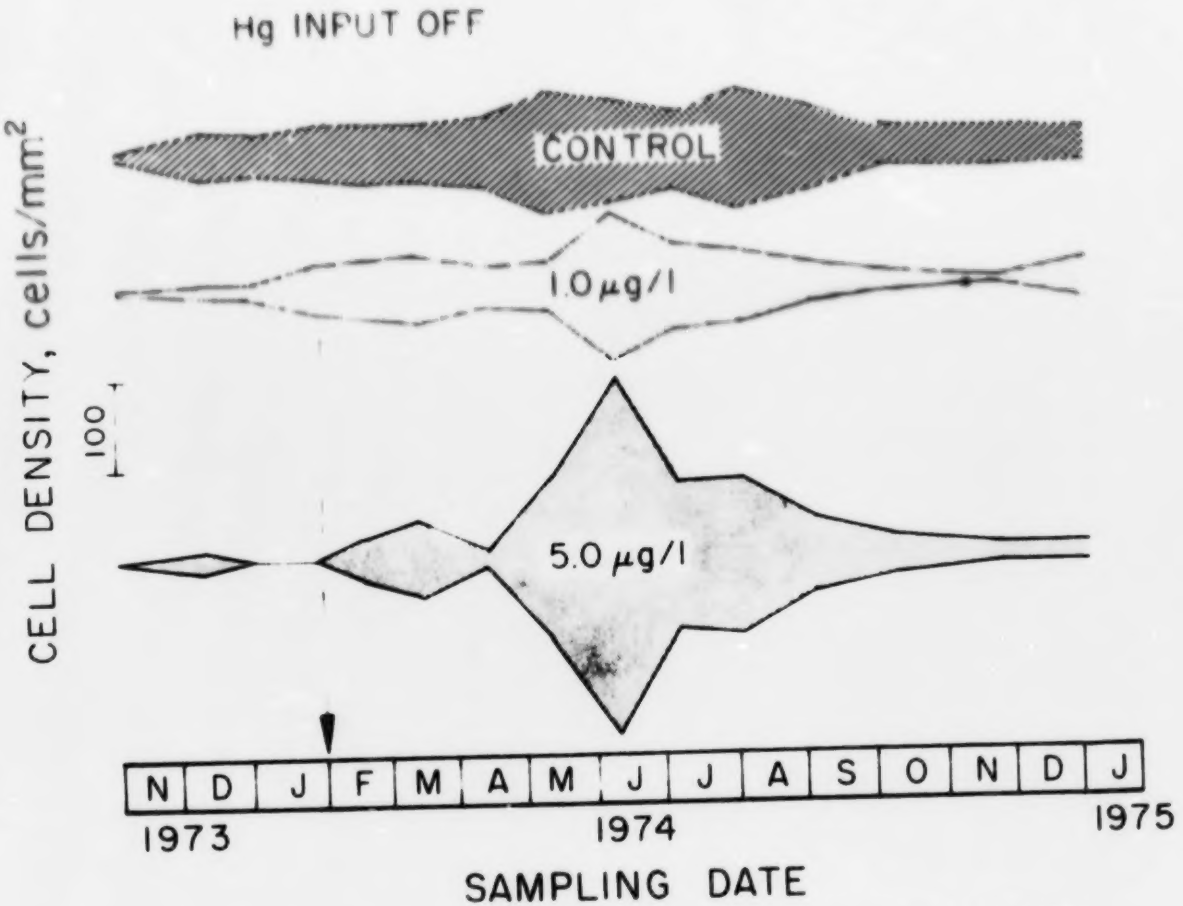

FIGURE 43. DENSITY OF *STIGEOCLONIUM ELONGATUM* ON PLEXIGLASS PLATES BEFORE AND AFTER MERCURY INPUTS WERE STOPPED.

channels 3 and 6 than in the other channels. At the end of the project, its density on plexiglass plates was similar in all channels.

Counts for this species from wall samples are summarized in Figure 44. The results are variable but indicate that S. elongatum was most abundant in the 5.0-μg/l streams. During the year after the Hg input was shut off, there was much less variation in the numbers of S. elongatum cells on this substrate than was seen on the plexiglass plates. This may have been due to the shaded location of the southwest-facing wall used for sampling.

Data for this species from the glass slides showed significant differences in cell densities due to treatment in the first two colonization experiments (ANOVA, $p < 0.01$) with reduced numbers in the Hg-treated channels. After the Hg was shut off, this reduction in initial colonization disappeared with a trend towards greater densities on the slides in the former Hg streams.

S. elongatum was isolated and tested under more controlled conditions with the CFC system. In the experiment in which the Hg concentrations were held relatively constant at 5.0 μg/l, the number of cells of this species was significantly reduced in the treated chambers ($p < 0.01$) compared to the controls. When the Hg input was shut off and clean slides were placed in the chambers, S. elongatum colonized slides in all chambers at similar rates.

Oscillatoria geminata - During the first summer of the study, this species was found to comprise numerically more than 10% of the algae inhabiting plexiglass plates in the streams, but during the next two years it was greatly reduced in abundance in the plexiglass plate and channel wall samples. It appears that O. geminata is well adapted for initial colonization and rapid growth on a new substrate but is unable to compete at later stages of succession.

This species exhibited a highly significant negative response to Hg treatment during the two glass slide experiments carried out just prior to the shut-off of mercury (ANOVA, $p < 0.01$). In the glass slide experiments after the Hg input was stopped, this treatment effect disappeared and O. geminata was found to be equally distributed in the streams. In two experiments with the CFC system this species was found to grow profusely in the controls and not to grow at all on the slides in the 5.0-μg/l chambers.

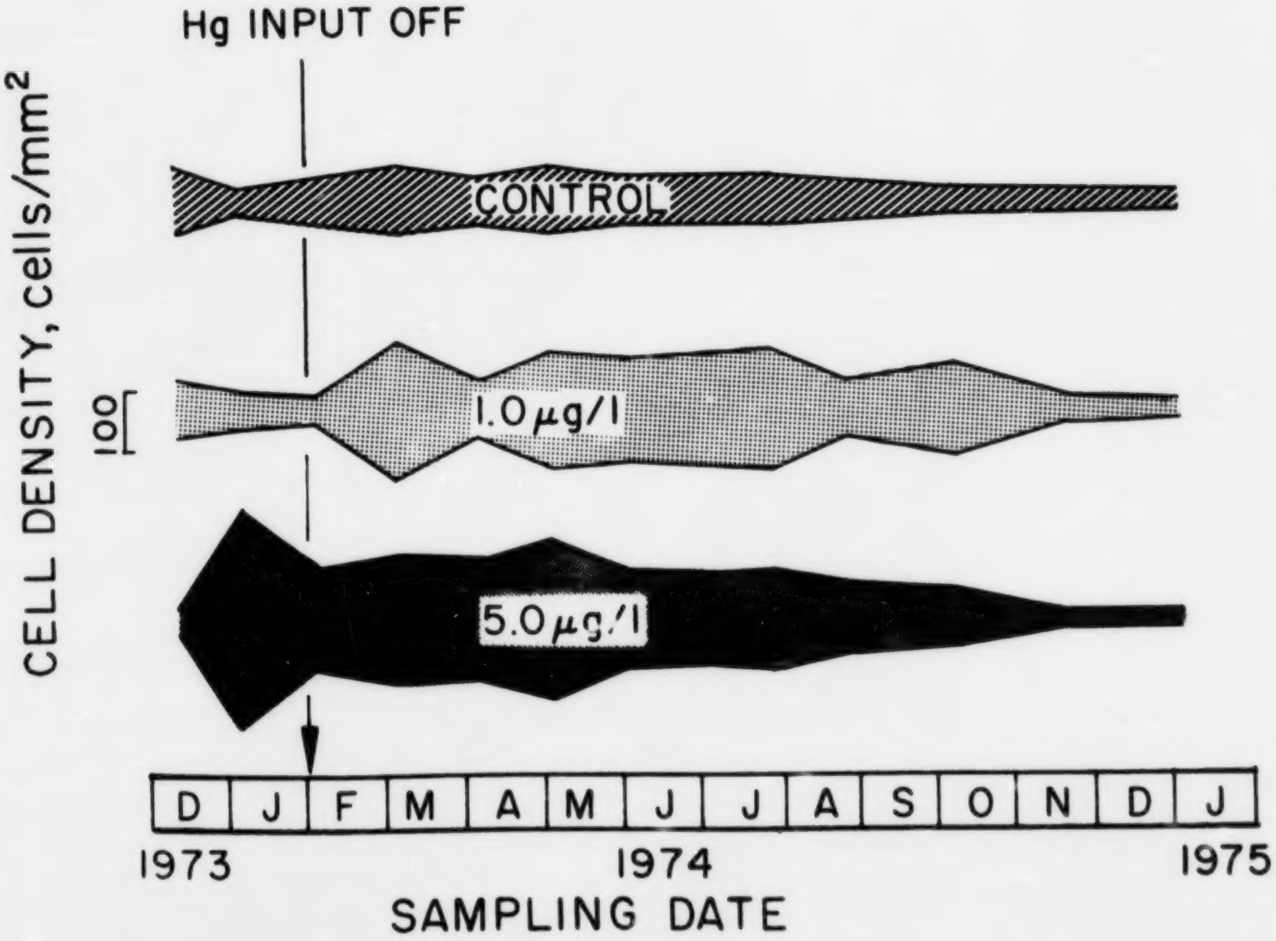

FIGURE 44. DENSITY OF *STIGEOCLONIUM ELONGATUM* ON CHANNEL WALLS BEFORE AND AFTER MERCURY INPUTS WERE STOPPED.

Chromulina pseudonebulosa - Although C. pseudonebulosa is flagellated, it was most often found on glass slides in a resting condition. During the early part of the Hg project, this species was observed to be most abundant on the plexiglass plates in the 1.0-μg/l channels (high level at that time) (Ferens, 1974); however, it did not appear to be common on this substrate in later samples. This species showed a significantly lowered colonization rate on glass slides in streams receiving a Hg input. In the glass slide experiment started one day after the Hg input was turned off, greatest colonization was found on the slides in the former Hg channels. In the last two microscope slide experiments this species was rare and showed no differences in growth between channels.

Geminella turfosa - There is no record of this species being observed in North America, but the relatively abundant filaments of narrow cells with spiral chloroplasts (Figure 45) resemble in every way the excellent description of Gloeotila turfosa in Sweden (Skuja, 1956). This species was later transferred to the genus Geminella (Ramanathan, 1964) and will be referred to by that name.

No significant density differences were seen between treatments for this species for plexiglass plates, walls, or glass microscope slides. There were, however, morphological changes in cell shape that were apparently caused by Hg treatment levels. Figure 45 illustrates normal and various abnormal cells observed on glass microscope slides during the input of Hg. Abnormal cells were often only slightly swollen but some appeared as spheres or branched cells. The numbers of abnormal cells observed during routine counts of glass microscope slides, both before and after the Hg was shut off, were recorded. Table 5 lists the relative abundance of abnormal cells observed for the various treatments and indicates a highly significant difference between the 5.0-μg/l treatment and controls and also between the two treatments while Hg was on. After the Hg inputs were discontinued, the differences disappeared and by the time of the last glass slide experiment (October, 1974) no more abnormal cells were observed. This species was never isolated into pure culture so laboratory experiments were not conducted. Working with three marine algae in axenic cultures, Nuzzi (1972) also found morphological abnormalities associated with Hg at concentrations in the growth medium equal to those in the artificial streams.

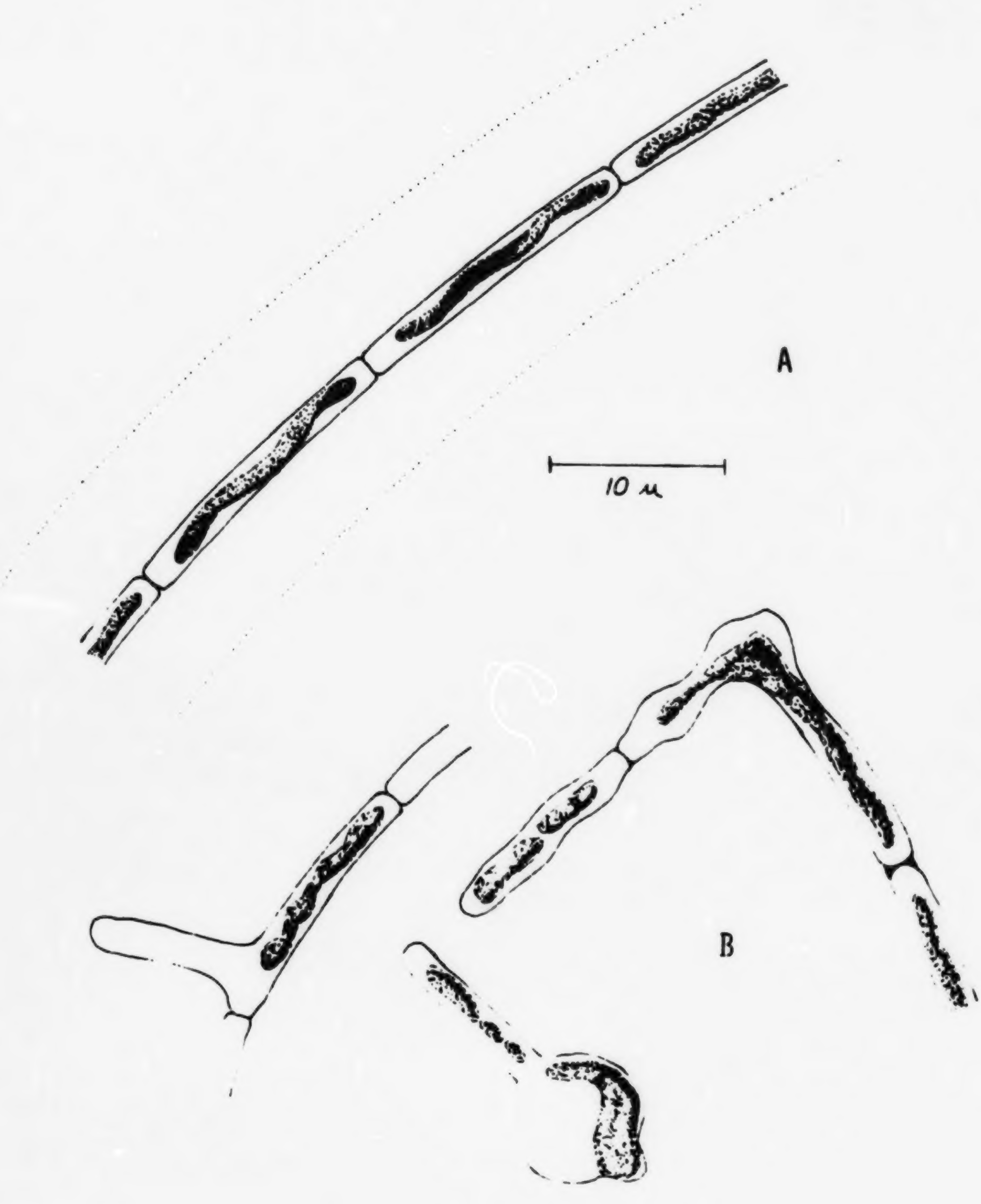

FIGURE 45. Geminella turfosa. A. Normal filament showing location of sheath, B. Various abnormal cells observed in mercury channels.

Table 5. INCIDENCE OF ABNORMAL *GEMINELLA TURFOSA* CELLS ON GLASS SLIDES DURING AND AFTER MERCURY INPUT (% OF TOTAL *GEMINELLA* CELLS)

During Mercury Input

	Treatment		
	Control	1.0 µg/l	5.0 µg/l
$\bar{X}$ ± 2SE	0.19 ± .24	1.16 ± 1.01	45.99 ± 10.40
n	21	24	35

After Mercury Input

	Previous Treatment		
	Control	1.0 µg/l	5.0 µg/l
$\bar{X}$ ± 2SE	0.42 ± .84	5.43 ± 5.42	3.84 ± 4.12
n	17	21	22

Microthamnion strictissimum - This branched green alga was unique because it was found to be common in the four Hg-treated streams, reaching its highest abundances on slides placed in the 5.0-µg/l channels, and was never seen in the control channels. This species was observed only during the winter of 1973 and showed a significant positive response to the Hg input level. These observations suggest that *M. strictissimum* might be an indicator of the presence of mercuric ions in low concentrations. This species was not isolated because it quickly disappeared after the Hg inputs were stopped.

Other species - Several other species of algae were more dense on the plexiglass plates and walls in the treated channels, and contributed to the higher total biomasses measured in these channels. *Cosmarium aspherosporum*, a small placoderm desmid that was common in all channels, was most dense in the controls (Figure 46 and 47).

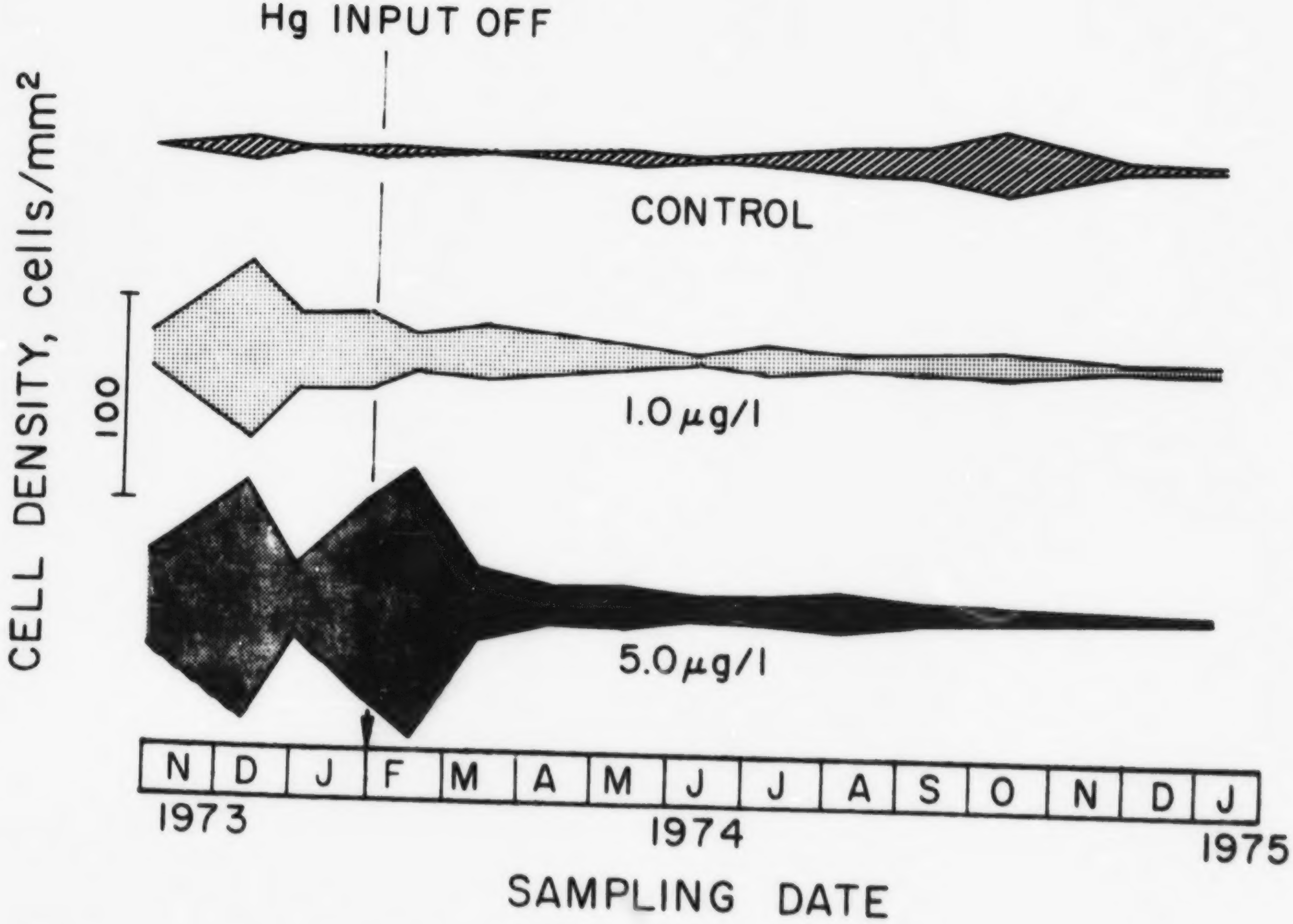

FIGURE 46. DENSITY OF *COSMARIUM ASPHEROSPORUM* ON PLEXIGLASS PLATES BEFORE AND AFTER MERCURY INPUTS WERE STOPPED.

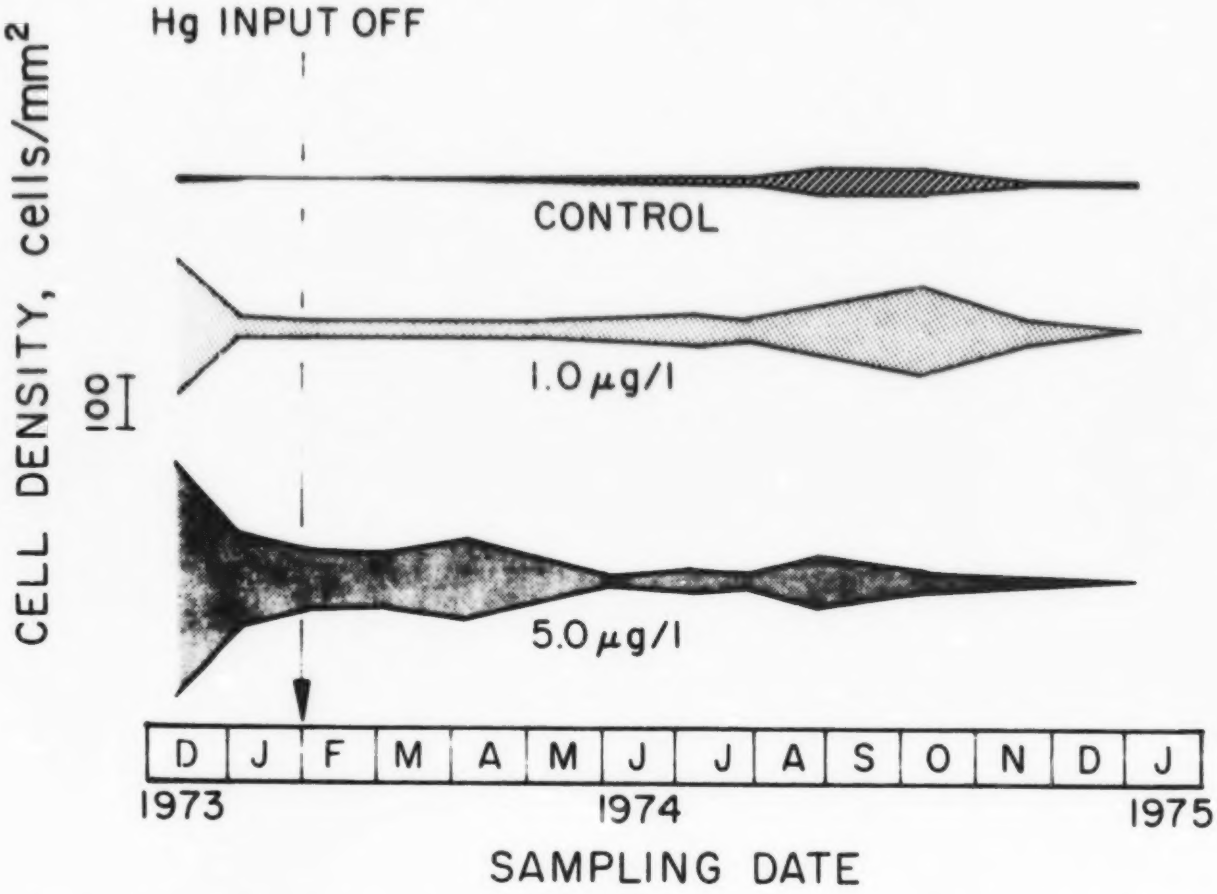

FIGURE 47. DENSITY OF *COSMARIUM ASPHEROSPORUM* ON CHANNEL WALLS BEFORE AND AFTER MERCURY INPUTS WERE STOPPED.

The density differences disappeared during the last year of the project. Spondylosium planum, a filamentous desmid, was also most dense in the 5.0-μg/l treatments and least dense in the controls while the Hg inputs were on (Figure 48 and 49). This pattern continued after the Hg inputs were terminated. In the short-term glass slide colonization experiments, both of these desmids showed greater growth in the treated channels with the highest numbers occurring in the 1.0-μg/l treatments.

Another species that was more dense on the plexiglass plates and walls of the treated channels was Merismopedia punctata, a blue-green alga of common occurrence. In the short-term colonization studies, however, this species showed no significant response to Hg levels.

Past studies concerning toxicity of Hg compounds to algae have all been short term in nature. These studies have shown various levels of toxicity by this metal to chlorophyll production (Hannan and Patouillet, 1972), primary production (Harriss et al., 1970; Knauer and Martin, 1972), and cell numbers (Ukeles, 1962; Nuzzi, 1972; Ben-Bassat, et al., 1972 and Matsui and Gloyna, 1972). Working with three species of marine phytoplankton, Nuzzi (1972) demonstrated reduced growth of all three species at a Hg concentration of 5.0 μg/l as $HgCl_2$. Knauer and Martin (1972) also found this same concentration to be inhibitory to marine phytoplankton. Our short term studies using the CFC system also showed this reduction in the initial growth of several fresh-water algae at a low concentration of $HgCl_2$; however, the study in the artificial streams demonstrated that an initial inhibition of growth may not always affect the long-term accumulation of a species.

The initial slow colonization seen in the Hg-treated channels seemed to be a result of a lowering of the reproductive potential of the algae and was related to the level of Hg in the water. The lower colonization rates were found in spite of denser periphytic communities on the older substrates in the treated channels, which should have provided larger seeding stocks of colonizers relative to the control channels. Apparently the cells that actually colonized a bare substrate such as a microscope slide were less common or less successful in the Hg channels. For some of the most abundant species such as Oedogonium or Stigeoclonium these would have been zoospores and for others they would have been merely drifting cells or fragmented filaments (Cosmarium spp., Oscillatoria, and Merismopedia).

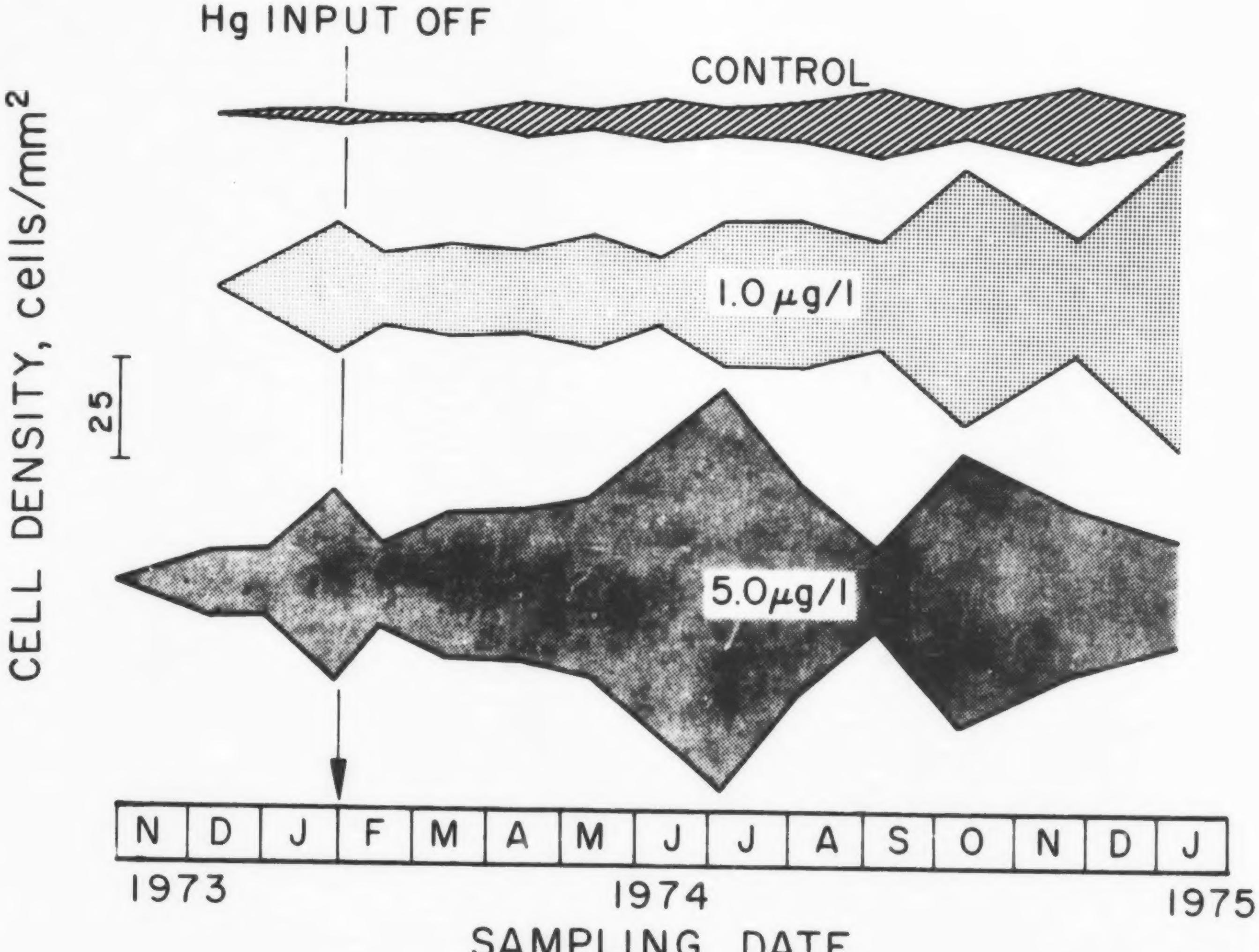

Figure 48. Density of *Spondylosium planum* on plexiglass plates before and after mercury inputs were stopped.

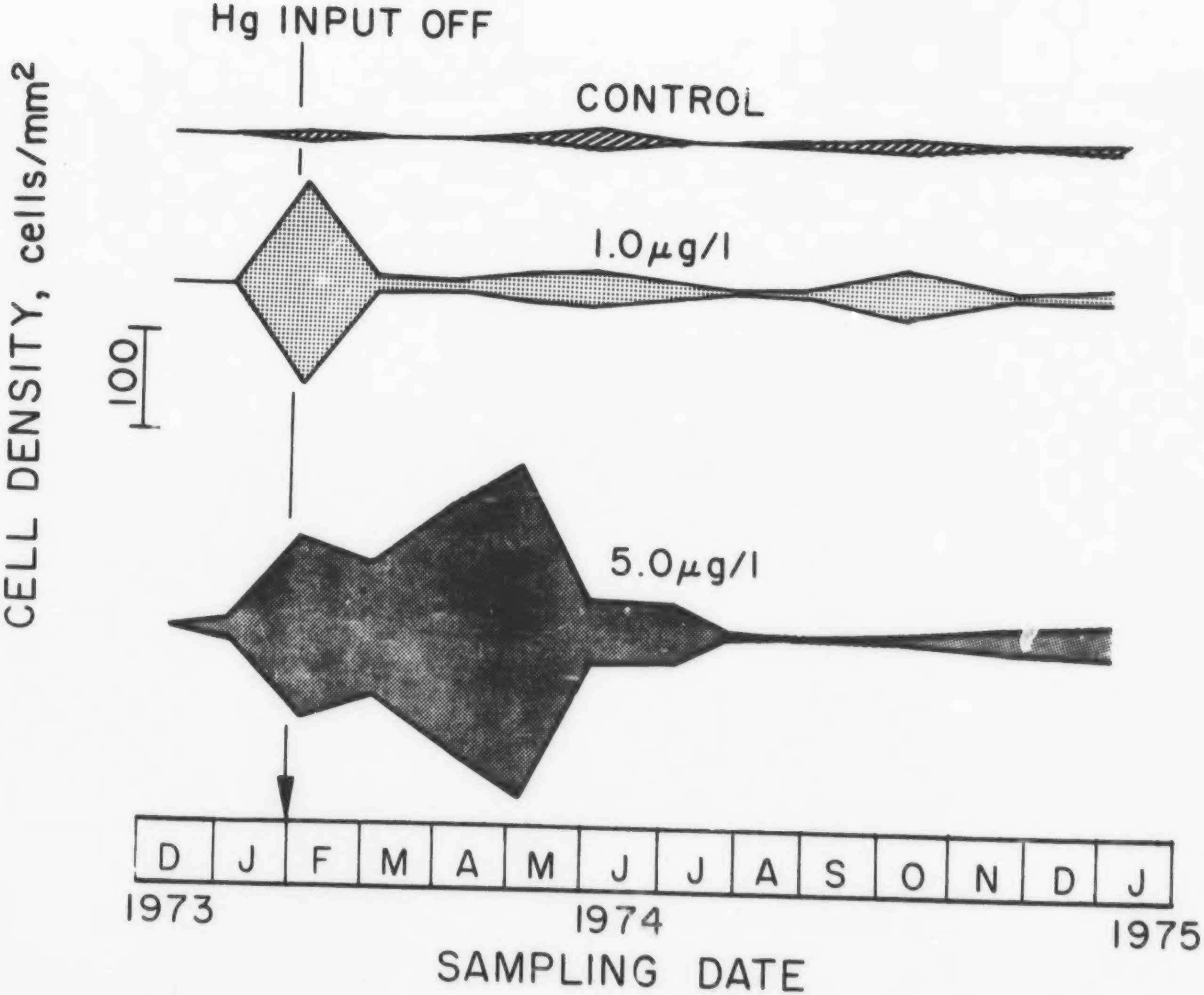

Figure 49. Density of Spondylosium planum on channel walls before and after mercury inputs were stopped.

The greater algal populations that were found in the Hg-treated channels may have resulted from nutrient level differences between channels, or they may have resulted from differences in consumer densities (including parasites and pathogens).

With respect to nutrients, the input of nitrogen with the Hg solutions never exceeded 0.5 μg/l, which represented an increase of 1.3% over background. This amount is probably not sufficient to cause the observed differences between control and treated channels. Nutrient stripping by macrophytes, especially in the dense stands that formed just below the input weirs of the channels, might have been an important factor. These macrophytic stands were much more dense in the control channels than in the treated, especially just below the Hg-input tubes. An enrichment of the water caused by death and decay of organisms coming into the channels from the head pools when these organisms were subjected to Hg inputs may have been a factor. Williams and Mount (1965) concluded that the higher periphytic biomasses they observed in Zn-treated channels were due to this mechanism. Unfortunately, personnel, time and equipment were not available for routine water analyses.

Little can be said with respect to consumer densities in the channels and their effects on periphytic growth. It has already been stated that tadpoles were established in the control channels long before they were observed in the treated. Dickman (1968) has shown that tadpoles enclosed in wooden cages severely reduced the standing crop of algae in proportion to the number of individuals present. Tadpoles may have been partially responsible for the reduced periphytic levels measured in the control channels, although this was not the only factor since glass slides suspended in the channels showed the same trends as the walls and plexiglass plates, and these glass slides were not grazed by tadpoles.

From the available information it is impossible to tell if the ruling influence on the differential development of algae in the streams was directly due to nutrient differences, grazing differences, a combination of these influences or some other factor dependent on Hg concentration.

Macrophytes

The vascular plants that became established in the channels were all typical of shallow ponds or very slow-moving water.

By the end of the study, *Juncus diffusissimus* and *Utricularia biflora*, were common in all six channels, although their densities were quite different between channels. The other three species of vascular plants found (see Appendix) were represented by very small numbers of individuals and seemed to be randomly distributed among the streams.

Early colonization and long-term biomass accumulation in the channels by vascular plants was dominated by *Juncus diffusissimus*, a species of rush of common occurrence in wet habitats of the southeastern coastal plain of North America. This plant was first observed in the streams nearly a year after water input was begun and by 20 months was well established in all of the channels (Table 3). It appears that the outside channels (especially channel 1) received higher inputs of seeds, and also that this plant was much more common at the heads of the streams than downstream. This increased abundance in the upper reaches of the channels became increasingly evident during the next 20 months until it was necessary to remove some plants to allow water to flow downstream.

Attempts to quantify the *Juncus* in the channels were not made until February, 1974. At that time the control channels were found to average 134 g/m^2 ash-free dry weight and the 1.0-and 5.0-μg/l channels averaged 60.3 and 62.5 g/m^2 respectively. The extremely poor growth of *Juncus* immediately downstream from the Hg input tubes showed that this plant was affected by Hg. Data collected during the last year of the project (with no Hg input) are summarized in Figure 50. There is no obvious explanation for the decrease in standing crop biomass of *Juncus* in the control channels except that this might be a normal successional trend, not seen in the treated channels because of the effects of the Hg.

The second most common plant by the end of the study was *Utricularia biflora* which bloomed during the spring of 1974. This species was not found to be abundant in any of the streams until after the Hg was shut off. Since no differences were observed between the control and treated channels, this appears to be another successional phenomenon. The other two species of flowering plants present, *Typha latifolia* and *Callitriche heterophylla*, were not observed to flower or fruit during the study.

Insects

All of the species of insects collected from the artificial streams were characteristic of sluggish streams or shallow,

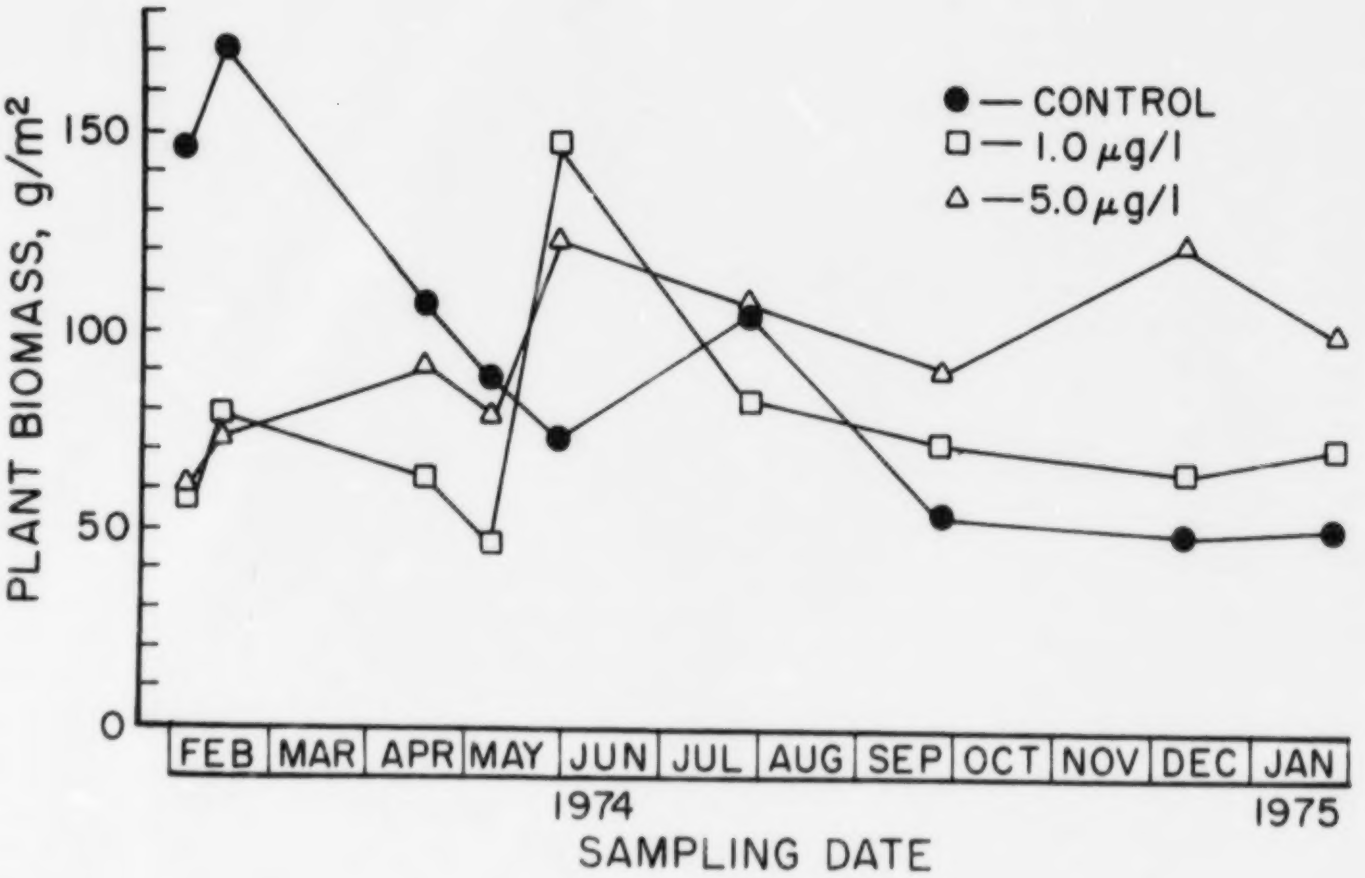

FIGURE 50. TOTAL MACROPHYTE BIOMASS (INCLUDING STEMS AND ROOTS) IN CHANNELS DURING THE FINAL YEAR OF THE PROJECT.

pond-type habitats of the southeastern United States. New species were continuously discovered in benthic dish samples throughout the three years of study indicating that colonization continued throughout this period. After 19 months of study, only 15 different species had been collected (Ferens, 1974). After 40 months of colonization, these original 15 species were still present but rare, and a total of 61 species had been encountered. Fifteen of these were first collected when the streams were cleaned out in February 1975.

The original species colonizing the streams were mostly midge larvae with one dragonfly, *Pantala hymenea*, present after the first year. By the end of two years another dragonfly, *Erythrodiplax minuscula*, and a damselfly, *Ischnura* sp., were present in addition to several new midge species. The first beetle larvae and adults were collected in January 1974, 29 months after input of water was started. At this same time the first true bugs, *Hesperocorixa* sp., were also collected. Four months later in May, 1974, the first tricopterans were found and in August 1974 the first aquatic lepidopterans were observed. As mentioned earlier, several species, including a helgrammite, a waterscorpion, several species of dragonflies and beetles and another lepidopteran, were not observed until the streams were cleaned out in February 1975.

During the initial part of the study when input concentrations were 0.01 and 1.0 μg/l, no significant differences in mean numbers and relative abundances of dominant species of midges were found (Ferens, 1974). Small but significant ($p < 0.05$) increases in diversity and evenness values were found for downstream samples from the 1.0-μg/l streams as compared to the controls; however, diversity values were very low during this time (about 1.0) because of the initially slow rate of colonization by insects.

Benthic dish data collected after the Hg inputs to channels 3 and 6 were increased to 5.0 μg/l in August, until the Hg was shut off six months later, indicated that insect densities were greatest in the 1.0-μg/l streams and very similar between the controls and the 5.0-μg/l streams (Figure 51). Fluctuations in these densities were observed to be similar between treatments for the year following the Hg shut-off, but statistical analysis showed that the density of insects was significantly lower (ANOVA, $p < 0.01$) in the previously treated streams. Diversity and evenness values for the benthic dish samples collected during this last year (Figure 52) were found to be significantly higher

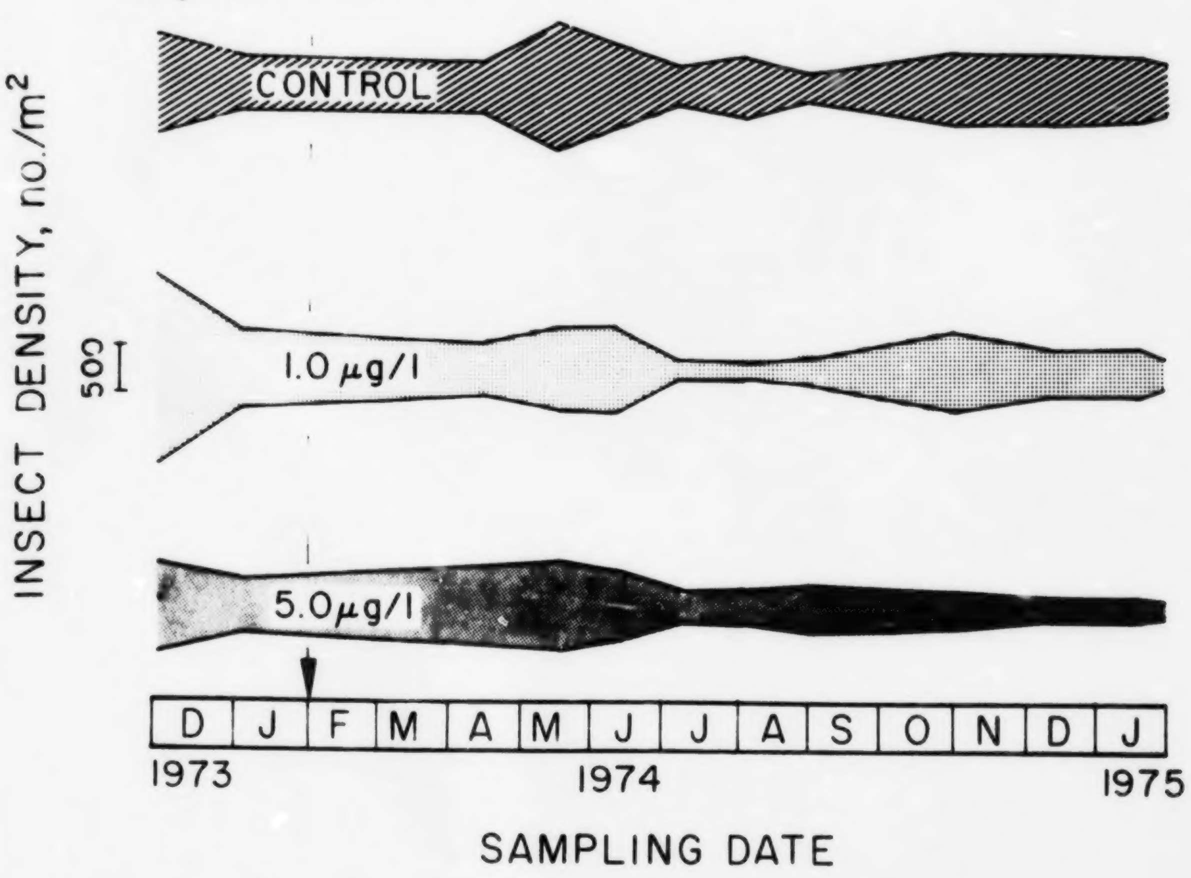

FIGURE 51. DENSITIES OF INSECTS COLLECTED FROM BENTHIC DISHES BEFORE AND AFTER MERCURY INPUTS WERE STOPPED.

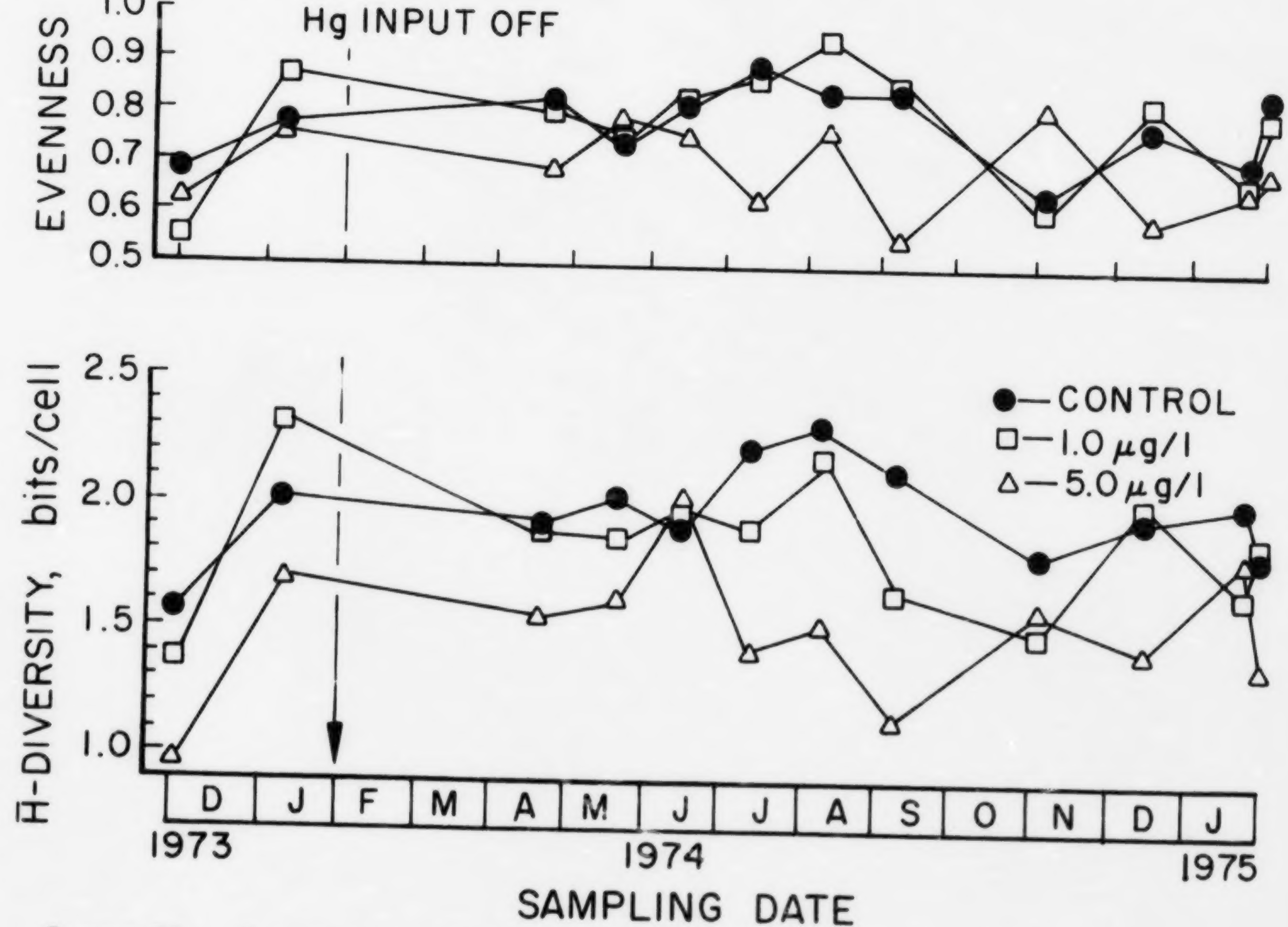

FIGURE 52. DIVERSITY AND EVENNESS OF INSECTS COLLECTED FROM BENTHIC DISHES BEFORE AND AFTER MERCURY INPUTS WERE STOPPED.

in the control streams than in the 5.0 μg/l channels (Students t, $p < 0.05$). This trend of lower diversity values for higher treatments was almost gone at the end of the summer after the Hg had been shut off.

Using ANOVA methods, three species of insects were found to be negatively affected by treatment levels in the streams. These were a damselfly, Argia sp., that reached considerable densities by the end of the study; a tiny mayfly, Callibaetis sp.; and one midge species, Paralauterborniella elachisa. Another mayfly, Caenis sp., was not collected in channels 3 or 6 during the input of Hg and this may have been related to the treatment. Three species of chironomids (Pseudochironomus sp., Ablabesmyia ornata, and Psectrocladius sp.) and one ceratopogonid (Dasyhelea sp.) were most abundant in the 1.0-μg/l streams.

When the water was turned off in February 1975, an attempt was made to remove the entire populations of the larger insects, amphibians, molluscs, and mosquitofish in the streams. The water level in the channels was lowered to about two inches above the sand. Starting at the tails, an 8.5-m stream section was enclosed with two screens, and ten passes were made with a 3-mm mesh dip net. The lower screen was then moved up 17 m thereby enclosing the next section. This was repeated until the entire 100 m length of each stream had been sampled. The material collected by this method was carefully examined in the laboratory and all animals were removed, sorted and counted. Representative organisms from each group were frozen for Hg analyses. Table 4 summarizes the data from this final sampling. The insect densities shown in Table 4 are much lower than those calculated from benthic dish data (Figure 51). This is because the sampling technique used did not collect midge larvae, the most common benthic insects. Table 4 shows that the greatest densities of larger insects occurred in channels 2 and 5, the 1.0-μg/l treatments, and the lowest densities in channels 1 and 6 (the first a control, the second a 5.0-μg/l treatment). There appears to be no relationship between treatment levels and diversity, density or evenness values.

Of the parameters measured for insects, diversity values appeared to be most sensitive to Hg levels with an initial increase in diversity for the 1.0-μg/l streams and an eventual lowering of the diversities corresponding to higher treatment levels. No significant differences were seen for insect densities while the Hg was on. In general the streams appeared to be variable with respect to insect numbers in a manner unrelated to treatment levels. This variation was probably the result of the finite number of non-random visits by egg-laying adults. None of the common species of insects occurring in the streams demonstrated a conclusive direct or indirect reaction to Hg levels, although possible inhibitory effects were seen for four less-abundant species.

Warnick and Bell (1969), studying the acute toxicity of various metals to three insect species, found Hg in the inorganic form to be lethal at 2.0 mg/l for all of the species tested. Of the three species, the mayfly was found to be most sensitive to the various metals. In our streams both mayfly species present showed a possible inhibitory response to a Hg concentration as low as 5.0 µg/l; however, this highest concentration tested has a very small effect on the total insect community.

SECTION VII

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SECTION VIII

APPENDIX

BIOTA OBSERVED IN THE ARTIFICIAL STREAMS

- Phylum Chlorophyta
  - Sub-Phylum Chlorophyceae
    - Order Volvocales
      - Family Chlamydomonadaceae
        - *Chlamydomonas* sp.
    - Order Tetrasporales
      - Family Gloeocystaceae
        - *Gloeocystis vesiculosa* Naegeli
      - Family Chaetochloridaceae
        - *Porochloris filamentorum* Pascher
    - Order Chlorococcales
      - Family Chlorococcaceae
        - *Chlorococcum humicola* (Naegeli) Rabenhorst
        - *Desmatractum bipyramidatum* (Chodat) Pascher
      - Family Oocystaceae
        - *Eremosphaera viridis* DeBary
        - *Oocystis* sp.
      - Family Scenedesmaceae
        - *Scenedesmus acutiformis* Schroeder
      - Family Coccomyxaceae
        - *Dispora crucigenioides* Printz
    - Order Ulotrichales
      - Family Ulotrichaceae
        - *Hormidium subtile* (Koetzing) Heering
        - *Geminella turfosa* (Skuja) Ramanathan
      - Family Microsporaceae
        - *Microspora pachyderma* (Wille) Lagerheim
        - *M. quadrata* Hazen
    - Order Chaetophorales
      - Family Chaetophoraceae
        - *Microthamnion strictissimum* Rabenhorst
        - *Stigeoclonium elongatum* (Hassall) Kuetzing
    - Order Oedogoniales
      - Family Oedogoniaceae
        - *Oedogonium* sp. (probably *O. pusillum* Kirchner)
    - Order Zygnematales
      - Family Zygnemataceae
        - *Mougeotia* sp. (5 micrometer width)
        - *Mougeotia* sp. (10 micrometer width)
        - *Spirogyra* sp.
      - Family Mesotaeniaceae
        - *Cylindrocystis brebissonii* var. *minor* West and West
        - *Mesotaenium macrococcum* (Kuetzing) West and West

- Family Desmidiaceae
  - *Arthrodesmus incus* (Brebisson) Hassall
  - *Cosmarium asphaerosporum* Nordstedt
  - *C. pseudoconnatum* var. *ornatum* Allorge
  - *C. laeve* var. *septentrionale* Wille
  - *C. undulatum* var. *minutum* Wittrock
  - *C. viride* var. *minor* West
  - *Penium spinospermum* Joshua
  - *Shaerozosma excavata* Ralfs
  - *Spondylosium planum* West and West

- Phylum Euglenophyta
  - Order Euglenales
    - Family Euglenaceae
      - *Euglena mutabilis* Schmitz

- Phylum Pyrrophyta
  - Class Dinophyceae
    - Order Dinokontae
      - Family Glenodiniaceae
        - *Glenodinium* sp.

- Phylum Chrysophyta
  - Sub-Phylum Xanthophyceae
    - Order Rhizochloridales
      - Family Rhizochloridaceae
        - *Stipitococcus vasiformis* Tiffany
    - Order Mischococcales
      - Family Pleurochloridaceae
        - *Chloroclostar minimus* Pascher
      - Family Sciadaceae
        - *Ophiocytium desertum* var. *minor* Prescott
  - Sub-Phylum Chrysophyceae
    - Order Rhizochrysidales
      - Family Rhizochrysidaceae
        - *Rhizochrysis* sp.
      - Family Stylococcaceae
        - *Lagynion macrotrachelum* (Stokes) Pascher
    - Order Chromulinales
      - Family Chromulinaceae
        - *Chromulina pseudonebulosa* Pascher
    - Order Ochromonadales
      - Family Ochromonadaceae
        - *Ochromonas* sp.
      - Family Dinobryaceae
        - *Epipyxis* sp.
  - Sub-Phylum Bacillariophyceae
    - Order Pennales
      - Family Eunotiaceae
        - *Eunotia tenella* (Grun.) Cleve

- Family Naviculaceae
  - *Navicula notha* Wallace
  - *Pinnularia abaugensis* (Pant.) Ross
- Family Cymbellaceae
  - *Cymbella* sp.

- Phylum Cyanophyta
  - Order Chroococcales
    - Family Chroococcaceae
      - *Merismopedia punctata* Meyer
  - Order Oscillatoriales
    - Family Oscillatoriaceae
      - *Oscillatoria geminata* Meneghini
      - *O. meslini* Fremy
      - *O. splendida* Greville
  - Order Nostocales
    - Family Nostocaceae
      - *Anabaenopsis* sp.
    - Family Rivulariaceae
      - *Calothrix parietina* (Naegeli) Thuret

- Phylum Spermophyta
  - Class Monocotyledoneae
    - Order Alismatales
      - Family Typhaceae
        - *Typha latifolia* Linnaeus
    - Order Liliales
      - Family Juncaceae
        - *Juncus diffusissimus* Buckley
    - Order Graminales
      - Family Poaceae
        - *Panicum* sp.
  - Class Dicotyledoneae
    - Order Geraniales
      - Family Callitrichaceae
        - *Callitriche heterophylla* Pursh
    - Order Scrophulariales
      - Family Lentibulariaceae
        - *Utricularia biflora* Lamarck

- Phylum Arthropoda
  - Class Arachnoidea
    - Order Araneae
      - Family Pisauridae
        - *Dolimedes sexpunctatus* Hentz
      - Family Lycosidae
        - *Lycosa helluo* Walckenaer
  - Class Insecta
    - Order Odonata
      - Family Coenagrionidae

- *Argia* sp.
- *Ischnura* sp.
- Family Libellulidae
  - *Celithemis fasciata*
  - *Epicordulia* sp.
  - *Erythrodiplax minuscula*
  - *Ladona deplanta*
  - *Pacydiplax longipennis*
  - *Pantala hymenea*
  - *Plathemis lydia*
  - *Tetragoneuria semiaquea*
- Family Aeschnidae
  - *Anax junius*
- Order Coleoptera
  - Family Dytiscidae
    - *Agabus* spp.
    - *Coptotomus* sp.
    - *Hydrocanthus* sp.
    - *Hydroporus* spp.
    - *Hydrovatus* sp.
    - *Laccophilus* sp.
    - *Suphisellus* sp.
  - Family Hydrophilidae
    - *Berosus* spp.
    - *Enochrus* spp.
    - *Tropisternus* spp.
  - Family Gyrinidae
    - *Gyrinus* sp.
- Order Ephemeroptera
  - Family Baetidae
    - *Caenis* sp.
    - *Callibaetis* sp.
- Order Trichoptera
  - Family Leptoceridae
    - *Oecetis* sp.
    - *Triaenodes* sp.
- Order Hemiptera
  - Family Veliidae
    - *Microvelia* sp.
    - *Velia* sp.
  - Family Notonectidae
    - *Notonecta indica*
  - Family Navcoridae
    - *Pelocoris femoratus*
  - Family Gerridae
    - *Gerris* sp.
  - Family Nepidae
    - *Ranatra* sp.
  - Family Corixidae
    - *Hesperocorixa* spp.

Order Lepidoptera
Family Pyralidae
Parapoynx sp.
Synclita sp.
Order Megaloptera
Family Corydalidae
Chauliodes sp.
Order Diptera
Family Tipulidae
Tipula sp.
Helius sp.
Family Chironomidae
Subfamily Chironominae
Chironomus spp.
Cladotanytarsus sp.
Cryptochironomus sp.
Dicrotendipes sp.
Microtendipes sp.
Parachironomus sp.
Paralauterborniella elachista
P. nigrohalterale
Polypedilum sp.
Pseudochironomus sp.
Rheotanytarsus sp.
Tanytarsus sp.
Subfamily Tanypodinae
Ablabesmyia ornata
A. peleensis
Larsia sp.
Zavrelimyia sp.
Subfamily Orthocladiinae
Cardiocladius
Cricotopus sp.
Psectrocladius sp.
Family Ceratopogonidae
Subfamily Dasyheleinae
Dasyhelea sp.
Subfamily Heleinae
Alluandomyia sp.
Palpomyia tibialis

Phylum Mollusca
Class Gastropoda
Family Lymnaeidae
Lymnea sp.
Family Physidae
Physa sp.

- Phylum Chordata
  - Class Teleostomi
    - Order Cyprinodontiformes
      - Family Poeciliidae
        - *Gambusia affinis* (Baird and Girard)
  - Class Amphibia
    - Order Anura
      - Family Bufonidae
        - *Bufo terrestris* (Bonnaterre)
      - Family Ranidae
        - *Rana catesbeiana* Shaw
        - *R. pipiens* Schreber
  - Class Aves
    - Order Coraciiformes
      - Family Alcedinidae
        - *Megaceryle alcyon* (Linnaeus)
  - Class Mammalia
    - Order Carnivora
      - Family Procyonidae
        - *Procyon lotor* (Linnaeus)